A Test of Future Planning Ability in the Rat

MSc (by thesis)

Gillian Stewart

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Abstract

The aim of this study was to investigate the planning abilities of nonhumans, specifically rats. This was assessed by the animals' tendency to behave in response to future rather than present motivations. For the purposes of this study the future motivation in question was anticipatory sensory specific satiety, i.e., the animals were trained to expect satiating exposure to a certain flavour of rat pellet in the near future. At the testing phase of the study the animals were offered an unexpected choice of two flavours prior to being exposed to the excess of the experimental flavour. This unexpected flavour choice consisted of the flavour that the animal was about to receive (the flavour *congruous* with the animal's expectation), and an alternative flavour, of equal familiarity and palatability (the *incongruous* flavour). The consumption of the congruous and incongruous flavours was recorded. When faced with this choice, an animal successfully anticipating satiation to the upcoming flavour would be expected to consume proportionally more of the alternative (incongruous) flavour, in order to maintain the pleasantness of the anticipated flavour. However the results were inconclusive: there was no significant difference between the proportion of the congruous and the incongruous flavours consumed, suggesting that the current group of animals was not capable of spontaneously anticipating the upcoming flavour. An altered procedure then investigated whether the animals were capable of *learning* to anticipate the upcoming flavour by introducing regular (and therefore expected) flavour choices. Under these new circumstances the animals consumed significantly higher proportions of the *congruous* compared to the incongruous flavour. Taken together, these results suggest both that the animals were unable to spontaneously anticipate being satiated by an upcoming flavour, and were unable to learn to anticipate this satiation following repeated trials. The results and certain assumptions of the study are discussed.

Chapter 1

Introduction

The Original Mental Time Travel (Memory based)

Mental Time Travel (MTT) (Suddendorf & Busby, 1997) was originally used to describe the ability of humans to 'travel' cognitively back in time to recall specific past experiences. It was this process that enabled episodic memory (Tulving, 1984), which could be considered a re-living of specific past experiences (episodes). This is distinct from semantic memory, which is memory for 'general knowledge'. For example, knowing Paris is the capital of France is a semantic memory, but remembering the specific occasion on which you learnt that fact – who told you, where you were, etc, would be an episodic memory and would have required a conscious 'travelling back' along one's own personal timeline. It is this kind of specific, vivid memory for past events that is unavailable to amnesic patients, and a gradual loss of this episodic ability is one characteristic of Alzheimer's disease (McKhann, Drachman, Folstein, Katzman, Price & Stadlan, 1984; Collie & Maruff, 2000). Consequently, there is much literature on human and nonhuman research on episodic and episodic-like memory, particularly surrounding the benefits of nonhuman research in this area to further understanding of neurodegenerative disease (Aggleton & Pearce, 2001; Morris, 2001; Hampton & Schwartz, 2004).

Mental Time Travel Encompassing the Future

Recent findings have provided an extra angle for such research however. The disorders mentioned above appear to show a deficit not only in memory (the ability to travel mentally 'back in time') but also in the ability to consider equally specific and vivid future occasions (travelling 'forward in time'). The cognitive decline of a person with Alzheimer's disease, for example, is marked by a simultaneous loss of the ability to either recall past episodes *or* to carry out the kind of forward-thinking that is necessary for managing finances or planning a meal etc, with these and similar activities becoming increasingly difficult (McKhann et al, 1984; Collie et al, 2000; Nedjam, Barba & Pillon, 2000).

Tulving (1984) considered this ability to plan for the future to be the main purpose behind humans' episodic memory ability. Although 'mental time travel' was coined after Tulving's 'episodic memory', Tulving considered that the kind of vivid, personal memory that he had tapped into could sensibly be thought of as the retrospective wing of an over-arching mental time travel system. In fact he considered the ability to 'relive' specific personal episodes to be a mere by-product of a system that was designed essentially to allow vivid projection into the future. He reasoned that mental time travel is cognitively demanding. It is necessary to generate an infinite number of possible future situations, evaluate the likelihood of each one, then put current needs on hold while action is taken to prepare for multiple future time points, all the while balancing these actions without compromising the wellbeing of the current self (Suddendorf & Corballis, 1997; Suddendorf & Busby 2005). This complex ability would only have been favoured by natural selection if it considerably increased our chances of survival. This means specifically being able to plan for an uncertain future, not to vividly relive the past. Of course it is important to have the ability to recall a dangerous past episode, for example, though its value only

comes with the ability to take steps to prevent a similar occasion in the future. If this is the case further research would not be confined to the area of memory but could encompass the ability to 'looking forwards' - the ability to plan for the future.

Evidence for a Single 'Dual Direction' MTT System in Humans

Recent functional MRI studies on healthy participants have demonstrated that thinking about past and future episodes evoke very similar patterns of neural activity, and in the same brain structures, which Botzung, Denkova & Manning (2008) consider provides evidence that the ability to envisage the past and future place are rooted in common cognitive resource. Patient studies too have investigated this: When considering amnesic patients for instance it does not come as a surprise that they are unable to recall past experiences, however recent research has demonstrated that these people are equally impaired when it comes to casting the mind forward – that is, amnesic patients are as unable to envisage specific future situations as past occurrences: Patient DB (Klein, Loftus & Kihlstrom, 2002) for example was asked to envisage himself carrying out activities that coming evening that would make him a good parent (he was in fact a parent). While he was able to name suitable generic activities 'make dinner, read a story' etc, he was not able to imagine himself personally carrying out any such tasks in the future, and hence could not provide any additional detail or make any preparations for carrying them out (picking up an extra loaf of bread on the way home, for example). DB 'knew' what parents were supposed to do; he just could not imagine personally doing it (Klein et al, 2002). Hassabis,

Kumaran, Vann & Maguire (2007) found similar results. As retrospective and prospective thinking appear to fail simultaneously after certain kinds of brain damage, it suggests that there *is* a 'mental time travel system' that encompasses both the retrospective and prospective elements of an individual's personal timeline.

These patient studies also demonstrate that 'future thinking' can be split into general semantic abilities and more specific episodic abilities just as memory can, suggesting once more that these abilities are connected. The patient described above had his semantic cognitions intact: he was able to give general 'scripts' of sensible future activities, just as many amnesic patients remain able to semantically recall a great deal of general knowledge. The impairment relates to recalling or envisaging anything specific and personal to themselves.

Further evidence for an MTT system encompassing both memory and future thought is provided by several developmental studies. In terms of language ability, children begin to use 'past terms' (*yesterday, used to, before*) and 'future terms' (*tomorrow,* if *I get thirsty, we* might) at around the same time (generally between 3-4 years old), suggesting a simultaneous grasping of the concept of their own personal past and future (Hudson, Shapiro & Sosa, 1995).

If these abilities do develop in tandem it would be expected that a child capable of reliably recalling specific memories (something already established) would also have the ability to express plans for specific future occasions. Suddendorf & Busby (2005) investigated both retro- and prospective abilities. Three, four and five year old children were asked what they did yesterday and what they wanted to do

tomorrow. The study found that a third of the three year olds, half of the four year olds and three quarters of the five year olds were successful in providing *both* a memory and a suggestion of a future activity that was sufficiently specific and personal. The similar retrospective and prospective figures for each age range suggest again that these abilities appear together.

Further evidence of young children's episodic versus semantic planning abilities comes from Atance & O'Neil's (2005) 'trip task.' Children of three, four and five were told they were going on a trip and asked to choose from a selection of items to take with them, explaining their choices. The older children appeared to choose items based on an envisaged future that was uncertain, using words like 'maybe' '*if* I get thirsty' 'just in case…' selecting items that they did not need at present but *might* need in a future situation, such as plasters, a bottle of water or an umbrella. Younger children gave answers more rooted in the present, such as choosing a book "to read" or a bottle of water "to drink" – similar to patient DB above, these children are able to call up a general mental 'script' of 'things that happen on a trip' (semantic type thinking), yet cannot invent a *specific* future scenario in which one item might be more useful than another – they cannot place *themselves* in the future (requiring episodic-like processes).

Hudson, Shapiro and Sosa (1995) also discriminated between semantic and episodic future-orientated abilities. Three, four and five year olds were asked to describe both generic plans (e.g., what happens when you go shopping?) as well as specific plans for their personal future (what will tom's party be like tomorrow?). The more generic questions required semantic knowledge of event 'scripts,' whereas the more specific required a genuine ability to think forward to a specific future occasion personal to them. While the former did not vary substantially across age groups (three year olds could call to mind general

shopping based activities nearly as comprehensively as the five year olds), the latter ability to genuinely 'think forwards' improved significantly with increasing age with the three year olds only rarely being successful, demonstrating a dissociation between these two kinds of future thinking, similar to the distinction between episodic and semantic memory.

These tasks rely heavily on verbal ability however, so tasks with a greater behavioural component should also be utilised in order to prevent confounding cognitive and linguistic abilities. Suddendorf and Busby's (2005) behavioural Room Task is one such planning paradigm that can be adjusted for nonhuman as well as human participants, though only humans were considered in the Suddendorf et al's experiment. It assesses the ability to behave in response to future anticipated needs rather than presently experienced needs. Three and four year old children were moved from a waiting room into a play room, and offered the opportunity to select a toy to accompany them into the latter room. In the playroom the children found a puzzle board missing its pieces. At test, one of the toy options offered to the child was the puzzle pieces. The same pattern of age-related abilities emerged for this task as those aforementioned: more four year olds were able to 'look ahead' sufficiently vividly to choose the puzzle pieces more often than any of the other items, which allowed these children to play in the otherwise empty playroom. The three-year-olds did not choose this 'correct' toy any more often than any other object, showing that these younger children were not able to think ahead in the same way.

The above studies suggest that between the ages of three and four comes an awareness of one's *personal* future. The four year olds were able to envisage future boredom in an almost empty playroom and successfully selected the toy that would help prevent it. The three year olds had not yet developed

the ability to consider future requirements that may be at odds with their current feelings (that they just liked the look of a different toy instead).

This is an important methodology as it is entirely behavioural. The behaviour shown allows an individual to balance present and future needs so that both the present and future self can be compromised as little as possible (Suddendorf & Busby, 2005). The 'consciousness' aspect of the ability is clear in humans because humans can talk about the process of thinking backwards and forwards and recognise that they are envisioning themselves at different points in time (Tulving 2001). However it has been argued that the expression of this self-awareness (the ability to report it through language) could be considered of secondary importance to the physical expression of behaviour that is based on anticipated rather than presently experienced motivations (Suddendorf & Corballis, 1997). It is this ability for future orientated *behaviour* that is presumably beneficial for mental time travelling individuals, not the ability to be conscious of doing it.

Suddendorf et al's (2005) aforementioned behavioural paradigm gives the same age-related results as other experiments that require mental time travel, which means this ability for future-orientated action can be taken as a behavioural marker of mental time travel abilities. It may be possible then that nonhumans are able to demonstrate this ability through behaviour.

Are MTT Abilities Uniquely Human?

If consciousness is not taken as a requirement, the possibility of behaviourally-expressed nonhuman mental time travel would suggest human and nonhuman cognition in this area was less disparate than originally thought. The creation of an animal model of mental time travel based on future-orientated action would allow increasingly detailed research on the neural pathways and mechanisms involved, greater understanding of which can only aid the development of surgical, drug, therapeutic or preventative treatments for human deficits in this area.

Those that consider mental time travel uniquely human are those that focus on self-consciousness as a necessity for action related to the past *or* future, for example Tulving (1984) considered that episodic memory was closely linked to self consciousness, stipulating that one had to be aware of re-living a personal past experience, which led him to suggest the ability to mentally time travel in either direction was exclusively human. In the absence of any striking evidence to the contrary reviewers generally agreed that this ability could in fact be regarded as such. Indeed following experiments in the 1970s, Bischoff-Kohler (cited in Suddendorf & Corballis, 1997) stated that nonhuman animals were in fact stuck in time and had no concept of a personal future *or* past independent of the drive states they were currently experiencing. Osvath and Gärdenfors (2005) saw particularly future planning (dubbed 'anticipatory cognition') as uniquely human and as evolving simultaneously with early human tool cultures and the need to transport animal carcasses over long distances and periods of time. In these circumstances the ability to mentally represent a distant future was a huge advantage (particularly if group members were capable of representing a future common to all).

Evidence for Nonhuman Retrospective MTT (episodic memory)

Despite the above emphasis on self-consciousness, numerous studies have put forward behavioural results considered consistent with episodic memory abilities in nonhumans - or episodic-*like*, to account for the absence of any agreed non-verbal markers of consciousness – (Clayton, Bussey & Dickinson, 2003; Eichenbaum & Fortin, 2003; Zentall, 2005; Schwartz, Hoffman & Evans, 2005).

Clayton & Dickinson (1998) put forward certain criteria for nonhuman behaviour that was deemed to show genuine episodic like ability: Any study must demonstrate that an animal is behaving in response to genuine recall of a complete, unique past experience (the 'what'. 'where' and 'when' of an episode) rather than acting on familiarity or learned semantic knowledge, that an animal can do so flexibly (i.e., can respond to changing test conditions), and can respond 'on the spot' to an unexpected test, rather than becoming familiar with the testing sequence which may result in them storing behavioural responses in advance and hence using semantic in place of episodic memory.

Clayton et al's (1998) scrub jays were able to demonstrate memory for *what* kind of food was cached *where*, and *when.* The birds could also respond flexibly when certain kinds of foods (what), or foods cached in a specific place (where) or at a specific time (when) were degraded by the experimenter, by seeking out those foods that were still good to eat. A study by Babb & Crystal (2005) utilised a similar task and demonstrated that rats were also capable of recalling what food was stored where after either a long or a short period of time (when). Eacott and Norman's (2004) episodic memory task for rats introduced the idea of 'what, where and which', to replace 'what, where when' considering that '*when*' and 'in *which* environmental context' (e.g., black or white, with differing textures) were simply alternative ways of differentiating past experiences. The rats were able to remember *what* sort of object had been placed *where* in *which* open-field context. To ensure the animals were not responding to simple familiarity in the open field the procedure was adjusted (Eacott, Easton & Zinkivskay, 2005) by

replacing this open environment with E-shaped mazes (of the same contexts as before, e.g., black and white with differing textures), such that the animals could not initially see round the corners to the objects and had to recall *where* (left or right) in *which* maze (black or white) held *what* object (novel or familiar). This the rats did successfully, demonstrating an integrated memory for what object, where, and in which maze context. Eichenbaum and Fortin (2003) also tested rats, demonstrating that the animals were able to recall the order in which odours had been previously experienced. Zentall's (2005) pigeons were able to report on their own previous behaviour (whether they had pecked at a stimulus or not) when asked to do so unexpectedly, though Schwartz, Hoffman & Evans (2005) argued that the intervals between the pigeons pecking (or not) and the unexpected test were too short to confirm that long-term episodic memory had been utilised. The same Schwartz et al (2005) attempted to show episodic abilities in King the gorilla, though the authors admit that due to the large number of similar trials King may have started to expect his tests and store event information semantically rather than episodically. His answers then would not have be based on genuine recall of a past event, but instead his 'knowing' which answer was right because he had purposefully committed it to memory previously, ready to relay it for a reward when asked. The study of Panzee the chimp (Menzel, 1999) had a similar problem: it was possible that her ability to inform her keepers where experimenters had hidden her food 16 hours earlier was based on her committing the information to semantic memory *as she watched them hide it*, rather than genuinely recalling the actual hiding episode when asked 16 hours later.

Despite the mixed results it is clear that at least some species and methodologies appear to show a positive result regarding behavioural indications of nonhuman episodic memory – Clayton et al's (1998) jays and Eacott et al's (2005) rats in the E-shaped maze. This would suggest that behavioural indications of nonhuman future planning should also be possible. If it could be established that nonhumans could behave in response to anticipation of a future event, with the displayed behaviour having no obvious

link to the animal's current needs, a future-orientated ability in nonhumans could be considered a possibility.

Evidence for Nonhuman Prospective MTT (future planning)

It is important to note that several studies *have* shown an impressive nonhuman ability to plan for the future, but based on current rather than anticipated future needs, which does not demonstrate a genuine future orientated ability. Julia the chimp (Doel, 1970; cited by Suddendorf & Corballis, 1997) is such an example. She was able to plan a complex order in which she should open a series of boxes, containing keys that opened yet more boxes, to ultimately receive a food reward. The authors suggest she is 'future planning' however Julia's behaviour does not necessarily suggest this ability because she was hungry and awaiting her reward while she was completing her task. Similarly Mulcahy & Call (2006) taught apes to use a tool that would remove grapes from a specially designed apparatus. The authors demonstrated that the apes could select an appropriate tool (when given a choice between tools that would work and tools that would not) and carry it with them (while they were taken into a waiting room and then *back* into the testing room) in order to use it. In some conditions there was an interval of 24 hours between tool choice and tool use which would suggest that these apes had cognitive access to at least 24 hours worth of future experience. Again, however, the apes selected their tool *while in the same room as the apparatus on which it was to be used* therefore they could still have been acting on a present rather than future desire to fish grapes from a box.

It should also be noted that many species do engage in effective anticipatory behaviour such as storing food for the winter (Suddendorf et al, 1997). This kind of behaviour does appear to suggest planning

based on future rather than current needs. Crucially however they are not future needs that are pre-experienced - young animals preparing for their first winter will store food that they do not currently need, though this will not be the result of mentally pre-experiencing the cold and food scarcity, they merely behave automatically along with every other member of the species, regardless of previous experience. Instinctive behaviour such as this is certainly future-directed, though by its very nature is inflexible and 'mindless', and must be so in order to guarantee survival.

However some studies do claim to show genuine nonhuman future-orientated cognition. Emery and Clayton (2001) suggest that scrub jays will deliberately re-cache food pellets if it is likely they will be stolen in the future. They compared the behaviour of the birds while they either cached in private or when watched by another jay (in the wild these birds will pilfer the food stores of others if given the chance and the same behaviour can be produced in the lab). It was thought that if birds were storing food with the *future intention* of retrieving it again, rather than just caching compulsively through instinct, they may alter their behaviour if they thought food they were presently caching might be at risk from future pilfering, and this was the case: Birds that were watched while they cached were more likely to re-cache their stores in alternative locations if given the chance. Several other manipulations make the results yet more interesting; the tendency to re-cache after being watched was *only* seen in birds that had previously pilfered another bird's store – i.e., they appeared to require the experience of thieving in order to take steps to prevent their own food being stolen. This demonstrates that while the ability to learn to re-cache is probably inbuilt, the actual behaviour only occurs after specific learning experiences. While it cannot be established whether the birds actually envisage a personal future in which they are disappointed to find their caches stolen, it is nevertheless a behavioural demonstration that they are capable of acting on an anticipation based on previous experience (Emery et al, 2001).

It could be argued however that the birds are still acting on current rather than anticipated future needs: it is likely to be *at the time* of their observed caching that they will register a feeling of 'these are not safe' (a general feeling of insecurity or unease). When they are then presented with the 'unsafe' tray again a few hours later the 'unsafe' feeling associated with this tray could initiate an instinctive drive to re-cache. This would not require conscious forward thinking, rather just a feeling of 'knowing' that things should not remain as they are. Observational evidence from wild birds might be more compelling. If a wild bird cached a supply of food and then voluntarily returned to the same site a short while later to re-cache *because* there had been other birds around at the time of the first caching, this would demonstrate something other than a familiar feeling of unease when re-presented with a tray. A wild bird that behaves as above would be putting current drives (foraging for yet more food, lining a nest, etc) on hold while it essentially 're-does' a previous job in order to protect future needs. It remains that Emery et al's (2001) jays change their behaviour based on previous experience *and* current circumstance (not all thieves automatically re-cache, it is only after being watched) to avert future disappointment, though it could still be considered contentious whether the birds are acting on needs that they are *not* currently experiencing.

As aforementioned, Zentall (2001) demonstrated that pigeons can report on their previous behaviour (peck or not) when unexpectedly asked to do so, suggesting a purposeful mental backtracking, or episodic memory. He also considers whether pigeons, and hence perhaps other nonhuman animals, can form 'anticipatory traces' as well as the retrospective 'memory traces' that allowed them to report on their previous behaviour.

The distinction is illustrated by a discrimination task in which one of two initial stimuli informs which of 2 subsequent stimuli is 'correct' and hence reinforced. Zentall's example uses shapes that cue colours – an initial triangle stimulus means that 'red' is the correct answer, while a circle denotes 'green' will be

rewarded. The question Zentall poses is, if there is a delay imposed between the shape stimulus and the colour choice, what method does the pigeon use to maintain the information in order to later receive a reward? Is it the case that the animal *remembers* the triangle until the red/green choice appears? Or does it immediately convert the triangle on seeing it to the answer 'red' and maintain this 'anticipatory' information until it is presented with the colour choice? Zentall investigated this question by considering the kinds of stimuli that make this task easier for the animals: colour cues appear to be processed more readily than diagrams of lines in various orientations, for example. Zentall found that performance increased when the initial cue was 'easy' to process, but did not alter when the end-point/choice stimuli was easy instead. Therefore the pigeons must be *remembering* the initial stimulus during the delay as it the 'easiness' of this stimulus that determines the performance gradient, hence the animals are not using future thinking here.

In further studies however Zentall (2005) demonstrated that pigeons *could* be encouraged to encode prospectively if experimental manipulations meant it became more efficient to do so, for instance if many initial stimuli corresponded to only two 'choice' stimuli it presumably becomes a better strategy to encode one of two 'answers' immediately than to try to maintain one of many 'questions' during a delay interval. Zentall sees this ability to code retro- and prospectively as an indication that nonhuman animals are not in fact stuck in time as the Bischoff-Kohler hypothesis states they are.

However, it must be considered that Zentall's pigeons were expecting rewards throughout the experiment (i.e., currently), and the delay timeframes were such that their behaviour may have been guided by working memory rather than a longer term and hence more stable retrospective or prospective trace. It is the ability to behave in anticipation of a future that is more than a few seconds distant that is a useful survival tool. It is an important finding that an animal normally coding

retrospectively can be encouraged to do otherwise, though longer timeframes would be more compelling.

One study that is particularly compelling is a recent effort by Correia, Dickinson & Clayton (2007) based on sensory specific satiety. This is the phenomenon by which extensive consumption of a particular flavour renders that flavour subsequently less pleasant compared to alternatives (Rolls, Rowe & Rolls, 1981). Correia et al's (2007) scrub jays were offered a choice of two foodstuffs to cache (A and B), and a short while later the birds were allowed to retrieve what they had cached. However prior to the caching stage, the birds were provided with extensive amounts of one of the two foodstuffs (e.g., A) to consume to satiety, and immediately prior to the retrieval stage the birds were provided with extensive amounts of the other flavour (B), to consume to satiety. Therefore if the birds were capable of anticipating the flavour that would be presented immediately prior to *retrieval* (B), they should cache the flavour that will be most pleasant to retrieve under those circumstances, i.e., flavour A. However, this would mean caching the flavour by which they had just been satiated, which should go against the birds' current drives of responding to sensory specific satiety. However the birds did choose to cache the food that went against these current drives, and in doing so demonstrate an ability to behave in a way that is orientated towards the future. Crucially also, this methodology was not repeated more than once. This means that the birds responded to a 'one-off' occasion, and therefore were not relying on the consequences of repeated previous occasions to guide their behaviour. This suggests a genuine ability to consider future needs above present ones.

A study that similarly attempts to demonstrate nonhumans choosing a course of action that appears counterproductive to their current motivations is Naqshbandi & Roberts (2006): Monkeys switched

their preference from a larger to a smaller pile of food when the choice of this smaller pile resulted in a water bottle being returned to the cage sooner. Crucially, the animals were not thirsty when they were making their large/small food choice, so they had not merely learnt to associate a physically smaller pile of food with the return of a desired object because at the time of choice this object was not desired. It appears that these animals could *foresee* a thirst that they were not presently experiencing, and with sufficient salience that their previous and intuitive preference for a larger pile of food was reversed. However the procedure was repeated several times, which introduces the possibility that the monkeys were learning from the consequences of their actions. They gradually shifted their behaviour from choosing a larger to choosing a smaller pile of food after learning that this resulted in a more agreeable and less thirsty future. This behaviour does not therefore fulfil the necessary unexpected and 'on the spot' decision which Griffiths, Dickinson & Clayton (1999) assert is necessary for genuine episodic-like memory, and as such genuine episodic-like future planning. It is still of note however that these monkeys were capable of *learning* to plan for the future. This is particularly interesting because the study utilised exactly the same procedure with rats, yet regardless of the number of repetitions or how subsequently thirsty the rats became, they did not switch their preference to the smaller food pile, and thus did not demonstrate they were capable of learning to anticipate a future occasion.

Other studies with rats have come to the same conclusion. McKenzie, Bird & Roberts (2005) used a radial maze with 8 arms in which rats were trained to store small morsels of either cheese or pretzels. After a delay the rats were allowed back into the apparatus to find and eat the food that they had stored, exactly where they had left it. In one experimental condition, the food stored in the arms on one side of the apparatus were consistently made inedible by the experimenters, so that when the rat returned, anything that had been stored in these 'degrade' arms was not good to eat. A strange result

was obtained – the rats learned reasonably quickly in which arms they should avoid *seeking* food on their return to the apparatus, as they left the degrade arms well alone. However they still continued to *store* food in these locations as much as in the other arms, even when there were fewer food morsels than arms and the rats could have eaten all pieces had they not stored them in the degrade arms. In the recovery condition the rats showed an intact *memory* for the locations in which food would be 'bad', though they did seem capable of looking ahead to this condition when storing food, i.e., they were *not* storing food with the intension of later retrieving it, as this would surely have resulted in avoidance of the 'degrade' arms during the storing condition.

The rat results are consistent with the Bischoff-Kohler hypothesis that animals cannot be shown to demonstrate behaviour unrelated to their current needs, i.e., that they appear to be "stuck in time". Some studies with jays and pigeons are interesting and show flexible use of foresight and the presence of an anticipatory memory trace respectively, however it is only Corriea et al's (2007) satiated jays and Naqshbandi et al's (2006) monkeys that give substantial pause for future thought. Are nonhumans really stuck in time or have we just not yet designed the behavioural tasks that will push them to travel through it?

The Present Study

The present study aims to design a methodology that will enable future-orientated behaviour to be demonstrated in rats. If successful this will be the first positive result of its kind, and will pave the way for continued research into this ability in the rat. Continued investigation may allow the formation of nonhuman and especially rat models of degenerative cognitive disease, aiding greater understanding of the area and ultimately the possibility of surgery or drugs that may postpone, relieve or prevent the debilitating symptoms of cognitive degeneration experienced by many humans.

Effective future *planning*, then, rather than self-consciousness, is the focus of this study on mental time travel. If participants are able to act flexibly in order to prepare for future needs that, crucially, *are not currently being experienced*, they could be considered to have an ability to mentally travel forwards to a specific point in their future even if, in the case of the nonhuman participants, they are not conscious of doing so.

The present study will attempt to guide the animals' behaviour by using anticipatory sensory specific satiety, similar to the aforementioned Correia et al (2007) experiment with jays. A task will be designed such that if the rats are capable of anticipating satiation by a particular flavour, their behaviour will be reflective of their future planning for this upcoming satiation.

Chapter 2

Sensory Specific Satiety - the groundwork

2.1 Introduction

This study is concerned with establishing whether or not rats are capable of future planning to the extent that they display behaviour that is clearly orientated towards the future, i.e., behaviour that only makes sense when considering the animals' future rather than present needs, as described by Atance & O'Neil (2001) and Suddendorf & Busby (2005) as behavioural criteria required for nonhuman future planning. The present study will utilise Sensory Specific Satiety (SSS). This is the phenomenon whereby an animal that consumes one particular flavour to satiation subsequently finds the same flavour (texture, smell etc) reduced in pleasantness compared to other flavours (Rolls, Rowe & Rolls, 1981). This study is concerned with establishing whether or not the present group of rats are capable of *anticipating* satiation to a certain flavour and behaving accordingly in response to this anticipation. This should mean that, given a choice between the anticipated flavour and an alternative, an animal will choose the alternative in order to preserve the future pleasantness of the anticipated flavour, as demonstrated by Raby, Alexis, Dickinson & Clayton (2007) who established this behaviour in scrub jays. If a rat can predict exposure to satiating amounts of flavour A, and is offered an A/B flavour choice, the preferred flavour should be the one incongruous with the flavour that is expected, i.e., a greater proportion of flavour B should be consumed. This will make the subsequent opportunity to become satiated by flavour A much more rewarding. If a rat does consistently demonstrate this anticipatory SSS it would suggest that there

is at least some rudimentary capacity for true future planning, as this incongruous flavour choice holds no *current* benefit for the animal, and only makes sense if expectations of future experiences are taken into account.

First however it must be established that the animals participating in the present study demonstrate normal SSS behaviour. This will be investigated in the following chapter, however in order to effectively test for SSS it is necessary to first establish the quantities and timeframes required to satiate a rat that is not food deprived (as none of the current animals are in this study, all having *ad libertum* access to food and water), and the preferences for various flavours of pellet. The SSS literature does not present a common result: some studies that require satiation simply provide a large excess of food over a relatively long period (3 hrs) in order to allow animals (in this case jays) to become satiated (Corriea, Dickinson & Clayton, 2007). Some studies involving rats have simply allowed *ad libertum* access to the usual cage food for 24 hours in order to satiate the animals prior to test (Petrovich, Ross, Gallagher & Holland, 2007), or providing rats with an excess of 7g of food to consume following 20 hours of food deprivation (same authors). Studies involving humans have ensured that human subjects eat nothing for 3-5 hours (or "since breakfast") and then were allowed to eat as much as they liked of certain satiating foodstuffs (Hetherington, 1996; Guinard & Brun, 1998). In terms of timeframe, Hetherington, Rolls & Burley (1989) found that the largest drop in pleasantness ratings in humans occurred within 2 minutes of a satiating meal being consumed. The same authors found that those foods that were most palatable were renewed in pleasantness within 1 hour of the food being consumed to satiety. In terms of nonhumans however there is no current literature detailing the minimum amount of exposure time and amount of food required for a rat to eat until satiated. This is not surprising, as several factors affect sensory specific satiety, for example Rolls & McDermott (1991) showed an effect of age, as elderly

subjects demonstrated reduced sensory specific satiety, whereas the phenomenon was far more marked in young subjects. Additionally the type of food consumed has an effect on SSS, with foods high in protein reducing more quickly in pleasantness than foods such as buttered roll and Coke (Vandewater & Vickers, 1992). Indeed Smeets & Westerterp-Plantenga (2005) show that the timeframe of SSS (citing the greatest decrease in pleasantness as occurring within 20 minutes) suggests that the phenomenon is largely based on sensory experience rather than the post-absorptive effects of the food consumed. All of this information is important with regards to the amount of food provided in order to bring about satiety in the current study: too little food will mean the animals do not experience satiety and so cannot possibly be expected to predict this state in later stages of the investigation, whereas using vastly surplus amounts of food is wasteful. With regards to timeframe, this information can be utilised when designing a task that allows satiety to be reached and yet remains efficient. These aspects of normal satiation will be investigated in experiment 1.

Also important are the flavours to be used. As before, the literature does not provide a convergent picture of which flavours may be universally preferred by rats, other than the well established preference across all species for sweet tastes and a general dislike for bitter flavours such as quinine (Berridge & Zajonc, 1991, and others). The present study will investigate the preferences of certain flavours with the intent of eventually selecting three equally palatable flavours for the study, denoted A, B and C. Two of these flavours (for example A and B) will be offered consistently as the standard flavour choice subsequent to satiation by any 1 of the 3 flavours A, B or C. For example, an animal is exposed to one of the flavours A, B or C in isolation, and is then exposed to the standard A versus B flavour choice. This allows for a simple test of SSS: following satiation to flavour A in isolation the B choice should be subsequently preferred, and vice versa. An animal satiated by flavour C in isolation need not (and in fact

should not) show a consistent A/B preference at choice. Correia, Dickinson & Clayton (2007) demonstrated result similar to this when testing their jays for SSS. The birds were provided with two foodstuffs at the same time – kibbles and pine nuts - on which to become satiated. When the birds were subsequently offered a flavour choice consisting of the same 2 foods neither one was significantly preferred. This is not precisely comparable with satiation to a relatively novel flavour or food (pretzels, for example), but the Correia et al (2007) result does show that if there is no satiation-based motivation to eat either of the foodstuffs when given a choice, then both flavours tend to be consumed in relatively equal proportions. Alternatively in this situation an animal may revert to whichever flavour is individually preferred by that particular animal, therefore any obvious individual flavour preferences should be noted. It is the intention however that the animals find each of the three flavours relatively equal in pleasantness, as a strong preference for (or avoidance of) any of the flavours may skew the results – particularly if the flavour/s in question is one of those offered at choice (A or B). For example an animal that prefers flavour B may consume this flavour whenever offered an A versus B flavour choice, regardless of the flavour just experienced, or that anticipated. Other (albeit human) literature has demonstrated that various levels of palatability have an effect on SSS and renewed flavour preference - Johnson & Vickers (1992) found that less-preferred foods dropped more quickly in pleasantness, and to a greater extent. The authors also demonstrate that the most palatable foods recover their pleasantness more quickly following satiation than less palatable foods. Therefore for the present study it is important that the flavours used are equally palatable, in order that the animals are motivated to choose which flavour choice to consume based on experience rather than intrinsic preference, and that the pleasantness of the flavours are reduced and renewed at an equivalent rate. Flavour preference will therefore be investigated in experiment 2 of this chapter.

Experiment 1

Investigating the timeframes and consumption of Satiation

2.2 Method

2.2a *Subjects*

The same subjects are used throughout the study. 10 experimentally naïve Dark Agouti male rats (Rattus norvegicus) were used. The animals were housed in one group of four and two groups of three in diurnal conditions consisting of 12 hour light/dark cycles (light from 7am – 7pm). The testing room was an approximately 60 second walk from the rats' home room and included an ascending flight of stairs. All tests were carried out after extensive habituation to this journey, until the rats' behaviour on entering the testing room was relaxed and curious. An elasticated white cloth entirely covered the cages during transportation to further reduce any stress. All testing was carried out during the light cycle (finished by 6pm at the latest) and the animals had ad libertum access to food and water throughout the study, their main diet of nutritionally complete pellets supplemented with sunflower seeds scattered amongst their bedding. All experiments were conducted according to the Animal (Scientific Procedure) Act (1986) and as permitted by the Home Office Project License.

2.2b *Apparatus*

A square enclosure measuring 100cm x 100cm x 40cm, made of wood and painted smooth grey on the inside, was positioned on a low table. Directly above this enclosure a camera was attached to a bar running parallel to the ceiling of the testing room, approx 2m above the enclosure. The monitor and DVD recorder to which the camera was attached were positioned approx 2.5m from the enclosure and faced away from it, such that the display could not cause a distraction for any animals in the enclosure. The experimenter sat as far as possible from the enclosure, behind the monitor. An angular desk lamp was placed on the floor, centrally behind the enclosure, its shade angled downwards and towards the wall to provide a soft, low light throughout the room. The windows in the door of the testing room were covered with black polythene. Three flavours of food pellet were initially selected: bacon, cinnamon and 'neutral'. Separate food trays were used for each of the pellet flavours to prevent scent interference – the trays were translucent white plastic lids from Tupperware-style containers, all identical and measuring approx 20cm x 20cm with a small lip (<0.5cm) running around the edge.

2.2c *Design*

All animals were exposed to an identical procedure. The initial phase of this experiment ran once a day for 12 days, the later stage ran once a day for a further 6 days. A rat was recorded as 'eating' when it was physically doing just that – not when simply facing, nosing, holding or otherwise engaging with the food pellets. The selected flavours were rotated such that after the initial 12 days each rat had experienced each flavour in the enclosure four times. Rats were always tested in the same order, 1-10.

2.2d *Procedure*

i) *Habituation*: Rats were initially habituated to the grey enclosure in groups of cage mates, such that rats 1-4 were placed in the enclosure together, followed by rats 5-7 and then rats 8-10, for 15 minutes on day 1 and 30 minutes on day 2. On day 3 the first cage was split so that rats 1 and 2 experienced the enclosure together for 30 minutes, followed by 3 and 4, and then followed again by the two cages of three rats. On the fourth, fifth and sixth days the rats were placed in the enclosure individually for 30 minutes, the lengthy habituation process reflecting the possibly intimidating nature of the large open enclosure.

ii) *Test*: A tray of pellets (approx 5g – considered an excess) was placed in a random location in the enclosure. The camera was started recording and rat 1 was placed in the enclosure. Rat 1 remained and was filmed in the enclosure for 20 minutes, after which the animal was returned to the home cage, the camera was stopped and the amount of food eaten by the animal was gathered by weighing. The pellet tray was then replenished so that it once again contained 5g of food, and was positioned in a different random location in the enclosure. The procedure was repeated with rats 2-10.

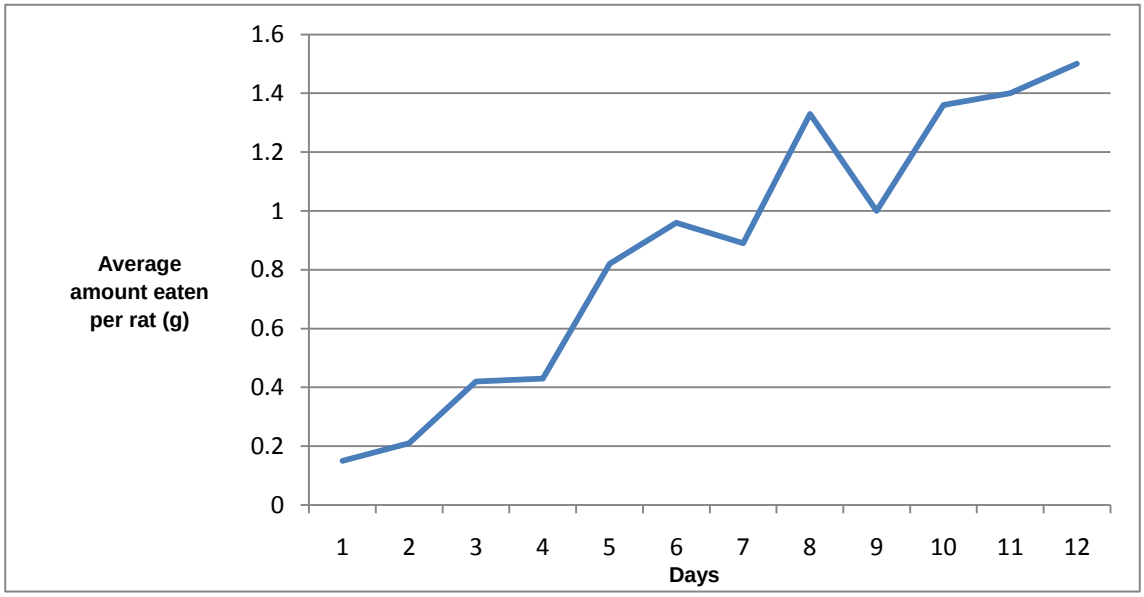
iii) *Data consolidation:* As above, the amount of food eaten by each rat each day was recorded. The DVD recordings were viewed by the experimenter. The amount of time (in seconds) that each rat spent eating during each of the 20 minutes was recorded, such that it was possible to establish when satiation occurred by noting which minute saw a drop in the amount of time spent eating. To plot a clear satiation curve the data was chunked into five four-minute time slots (to give the number of seconds spent eating during minutes 1-4, 5-8 etc).

2.3 Results

Amount of food eaten

As shown in fig 2.1, the average amount eaten per day rose fairly steadily from day one (0.13g) to day 12 (1.48g), as the rats became habituated to eating in the enclosure, and to the pellets themselves.

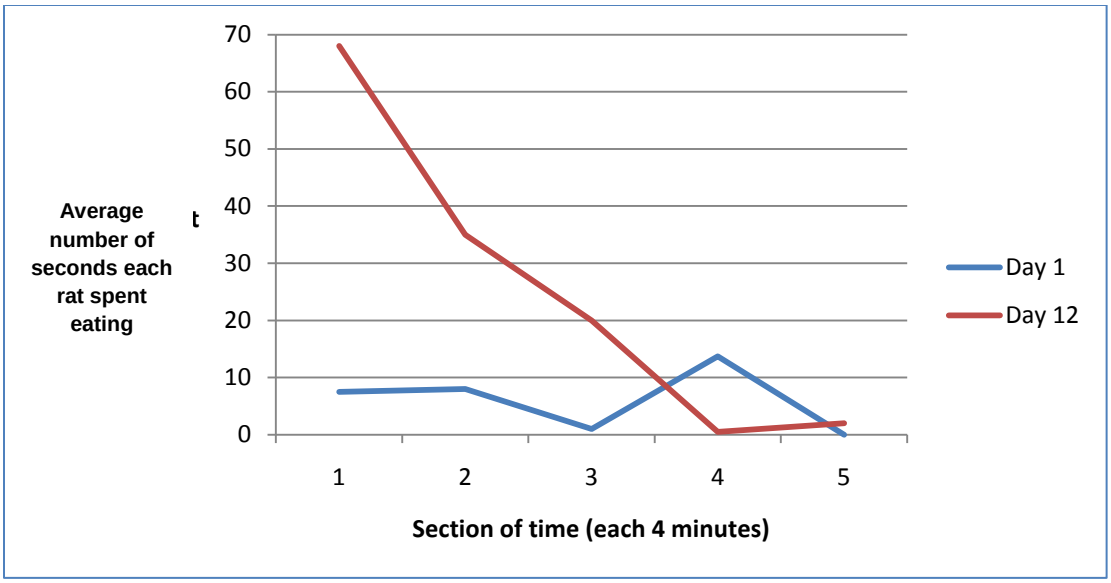
Fig 2.1: Average amount of flavoured pellets eaten per rat / per day, over 12 days



Satiation Period

Fig 2.2 below shows there is an obvious habituation period while the rats become accustomed to eating in the enclosure, as during the first few days the average amount of time the animals spent eating per 4-minute chunk of time is low (on average never reaching even 20 seconds eating time) and relatively erratic, with no obvious peak and subsequent drop-off, as demonstrated by the blue line on fig 2.2 which shows the animals' behaviour on day one. At this point the rats are not being satiated as the characteristic reduction in pleasantness is not seen (Rolls, Rowe & Rolls, 1981). However by the final days of the animals' exposure to this environment (day 12, shown by the red line on the above graph) there is a more obvious favoured time period for eating which begins on entering the enclosure (68 seconds within the first 4 minutes) and drops to almost half this value by 8 minutes (35 seconds spent eating between 4 and 8 minutes). This demonstrates the characteristic waning of interest due to a reduction in the pleasantness of the flavour being consumed, i.e., the animals have consumed this particular flavour to satiety. The average overall amount of time spent eating is also much higher at this later stage (day 12 as compared to day 1, for example), with the animals eating for over a minute in the first four minutes on day 12. By this point the rats are fully habituated to the enclosure and to eating in it.

Fig 2.2: Comparing the satiation curves of day 1 (blue) and day 12 (red) - the average amount of time each rat spent eating per four-minute span during 20 minutes in the enclosure


Average number of seconds each rat spent eating
70
60
50
40
30
20
10
0
1
2
3
4
5
Section of time (each 4 minutes)
Day 1
Day 12

2.4 Discussion

Amount of food consumed

The individual amounts eaten by each rat varied substantially, though the eating of all the animals increased with their experience of the enclosure. The average amount eaten by the end of the 20-minute trial was 1.48g. Only twice did a rat (on both occasions rat 6) eat more than this, consuming 2.1 and 2.6g on days 11 and 12 respectively. As this was a considerably rare occurrence it was considered that 2g of food would be sufficient to satiate this group of animals. It was not considered appropriate to present differing amounts of food to individual rats based on individual consumption rates, as larger or smaller amounts of food may have affect the animals' baseline motivation to eat – i.e., studies with rats have demonstrated that a larger pile of food is consistently preferred over a smaller pile (N and Roberts, 2006). For this study it was considered that individual motivation for consumption of food should (as far as possible) be kept equal across the group.

Timescale of satiety

Given 20 minutes to explore the large enclosure and eat flavoured pellets at will, the rats on average dramatically reduced their initial consumption rate during the second 4 minute section of time, i.e., between 4 and 8 minutes. However as seen from figure 2.2 above, the rats did not eat constantly while in the enclosure – even during the initial 4 minutes in the enclosure, just over 1 minute was spent physically eating the pellets, with the remaining time spent exploring the enclosure. This suggested that the amount of time allowed for satiation here was not necessary, and that it should be possible for the animals to become satiated within a reduced amount of time. If given less time in the enclosure the rats

may learn to reduce their time spent exploring in favour of a greater initial amount of time spent eating, resulting in swifter satiation. This would mean that any subsequent satiation tasks could be designed more efficiently, and the possibility was therefore investigated in the experiment below.

Experiment 1 (part 2)

Investigating satiation at five minutes

2.5 Method

2.5a *Apparatus*

This remained identical to the previous experiment.

2.5b *Design*

This was identical to the above experiment apart from the reduced number of trials; six rather than 12 were carried out.

2.5c *Procedure*

This was identical to the above experiment, except that the animals were removed from the enclosure after five rather than 20 minutes. This produced much shorter DVD recordings of the rats' behaviour, such that the experimenter now 'chunked' the time spent in the enclosure into five one-minute periods, so that the resulting graphs show directly how many seconds were spent eating every minute.

2.6 Results

Average overall amount of food eaten

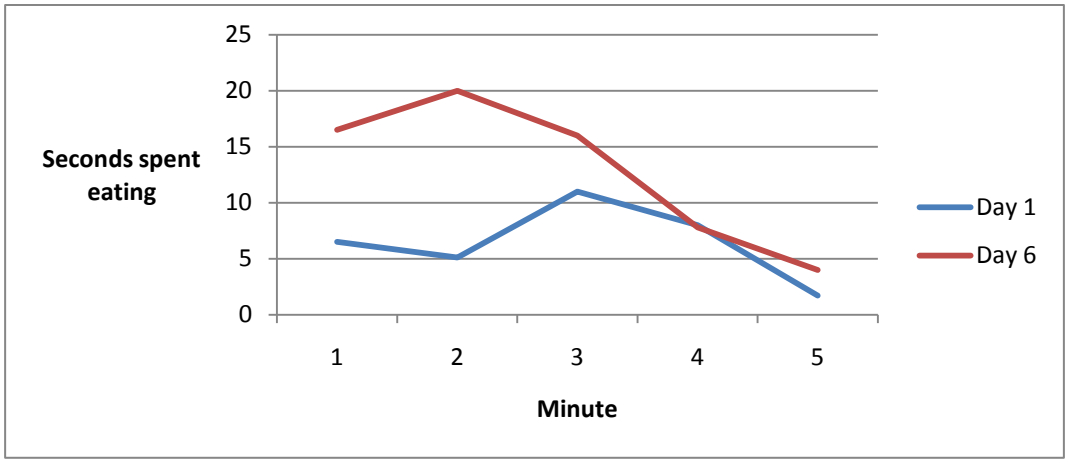
On day one of the five-minute-exposure trial the average amount eaten was 0.47g, considerably down on the 1.48g of the last day of the 20-minute-exposure trials. Over the next two days however it appeared that the rats learnt that they would be removed from the enclosure sooner than before and began to eat greater amounts within their new five-minute time span, the average rising to 1.01g by day three (however over half the rats were eating above 1g). Unfortunately a weekend fell halfway through this six-day run of testing, resulting in the rats appearing to 'forget' the enclosure/pellet scenario and regress in terms of how comfortable they were in the testing environment. This caused a substantial

drop in the amount of food eaten, reducing to an average of 0.17g on day four. However as before the animals appeared to learn quickly and by day six the average amount eaten was up to 0.94g, again with half of the animals eating 1g or well above it.

Timeframe of satiation

As with the first few days of the 20-minute trials, the amount of time spent eating as the 5 minutes progressed was initially erratic (shown by the blue line on Fig 2.3 below), not showing the characteristic peak followed by a waning of temporally-assessed interest. However by day 3 of the five-minute trials there was a recognisable curve in place, showing a substantial reduction in eating time after about 3 minutes. The aforementioned unhelpfully-placed weekend did not appear to have a large effect on the established satiation curve, with the three-minute point still marking a reduction in eating time even though the amounts being eaten were smaller than before. The shape of the satiation curve remained intact from day 3, such that by day 6 (red line on fig 2.3 below) the only change had been an increase in the overall amount eaten. The consistency of this curve suggested that as long as the animals were accustomed to the five-minute condition, they reached flavour-based satiety after about three minutes of exposure to the appropriate flavour.

Fig 2.3: Comparison of satiation curves on day 1 and day 6 of five-minute exposure, averaged across rats



2.7 Discussion

It appears possible to satiate rats to a certain flavour in 3 minutes, though to allow for a small margin of error a five minute exposure period to all subsequent flavours and contexts will be used. This allows sufficient time for flavour satiation yet is more efficient than 20 minutes per rat and, importantly, does not compromise the amount eaten as even though the average amount eaten during the 5 minute trials is less than that eaten over the 20 minute trials (1.01g compared to 1.48g), the 20 minute trials commonly produced a second surge of eating near the end of this allotted time. This result supports investigations by Hetherington, Rolls & Burley (1989) into the timeframe of SSS, which show that rats begin to resume their consumption of a satiated flavour within 20 minutes of the first initial reduction in pleasantness. With regards the overall amount eaten, the amount consumed within a 20 minute trial would therefore include post-satiety 'resumed' consumption, which did not contribute towards the

animals' initial satiation. Therefore the reduction in average amount eaten from the 20 minute to the 5 minute trials was not considered acceptable. Additionally, as the five-minute time period does not allow for this resurgence in pleasantness it is even less likely that the animals would eat in excess of the designated 2g, confirming this as a sufficient amount of any flavour to bring about initial satiety to it.

However the flavours and pellet types used in the above experiments were only samples and not available for any subsequent work, therefore the study was now concerned with habituating the animals to the new pellets and the contexts to be used, establishing flavour preferences, and beginning to pair the new flavours with the contexts in order to develop context-flavour associations which could then be utilised to test anticipatory sensory specific satiety.

Experiment 2

Establish flavour preference

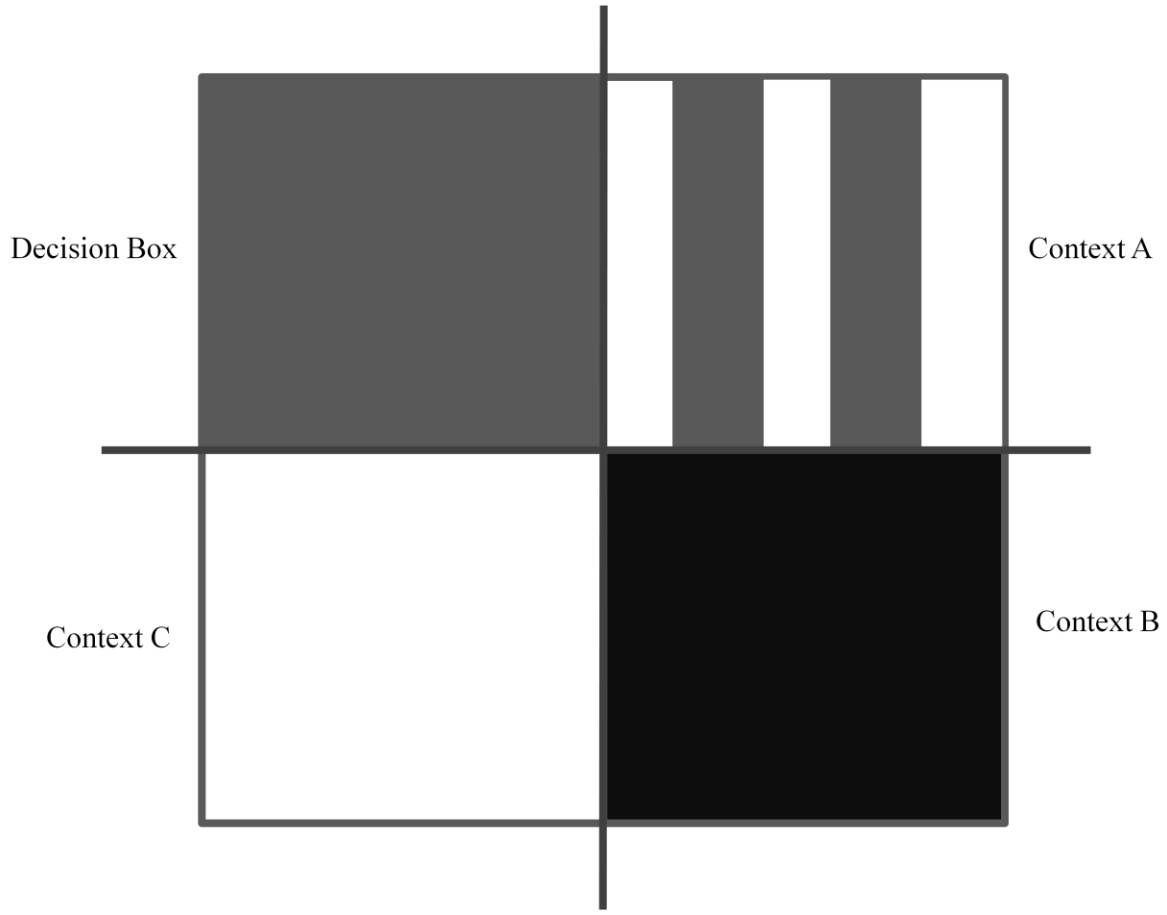
2.8 Method

2.8a *Apparatus*

The 100cm x 100cm enclosure previously mentioned was divided into four equally sized square compartments, each measuring 0.25m x 0.25m (see Fig 2.4). The thin boards of wood used to divide the

enclosure measured 60cm high to ensure the animals were not able to jump or look over this partition. Three of the compartments became differently coloured/textured *contexts* - A, B and C. Context A was striped with a sturdy wire mesh floor (mesh was square and 1cm^{2}to ensure that it would not trap the animals' feet). Context B was painted entirely black with a transparent Perspex floor. Context C was painted entirely white though no tactile floor was added. The fourth compartment remained the colour and texture of the original enclosure, i.e., painted grey.

Fig 2.4 The apparatus used, demonstrating the division of the original large enclosure into 4 separate compartments



This apparatus remained the same distance from the floor and the camera as before and identical food trays, lamp, etc were used. The new food pellets were "Omnitreat" flavoured pellets, each weighing 45mg and available in the following six flavours: chocolate, banana, peanut butter, tropical, pina colada and grape.

2.8b *Design*

Flavours were paired with the afore-described contexts as follows: context A = chocolate (henceforth 'flavour A' etc), context B = tropical, context C = peanut. The fourth (grey) compartment would be the neutral location for offering a flavour choice in later experiments, and this would always comprise the same two flavours, initially selected to be chocolate and tropical (A and B). It was considered that any flavour *choice* should be offered in a relatively 'neutral' environment, as presenting a flavour choice in a compartment already associated with a certain flavour may result in choices being made based on simple familiarity or novelty seeking rather than SSS (i.e., an animal may choose a flavour simply because it is the 'correct' flavour for that context, or alternatively may choose the alternative because it is not and therefore more interesting). Based on the previous experiments all contexts would be experienced for five minutes, and all food would be presented in amounts of 2g. The design of this

experiment serves to compare flavour preferences, associate contexts with flavours, habituate the rats to both the new food and the new environment, and to habituate the rats specifically to having a food *choice* in the decision box. It should be acknowledged that three contexts (and therefore three accompanying flavours) were utilised here rather than the two that would be strictly necessary to investigate sensory specific satiety (the 'same' flavour and a 'different' flavour). An extra context was used here in order to provide scope, should it become necessary, to investigate food choice prior to, or following, a 'neutral' context / flavour. The extra context / flavour would also serve to increase the number of flavours with which the animals were familiar, which increases the chance of finding two flavours that are equally palatable for use in the decision box.

In the initial habituation period the food trays were present in the contexts, though the food was not. It was considered important to expose the animals to just one entirely new aspect of the procedure at a time, and the contexts were an easier starting point than the flavours in isolation. Also this meant that from the animals' very first experience of the new flavoured pellets they may begin to associate them with their respective contexts, with which they would already be familiar. It was not considered necessary to habituate the animals to the contexts in pairs, based on the rats' previous experience of the testing room and also due to the contexts being smaller and therefore less threatening than the original 100cm x 100cm enclosure. Due to the partitions between the contexts being sufficiently high to prevent animals moving or looking from one context to another, up to four animals could be habituated at one time, one in each section.

2.8c *Procedure*

Habituation

Each rat experienced each context for 10 minutes, twice, on day 1 of habituation and 20 minutes, once a day, on days two and three. By the end of this third day of habituation all the rats appeared comfortable in the different contexts, therefore testing could begin.

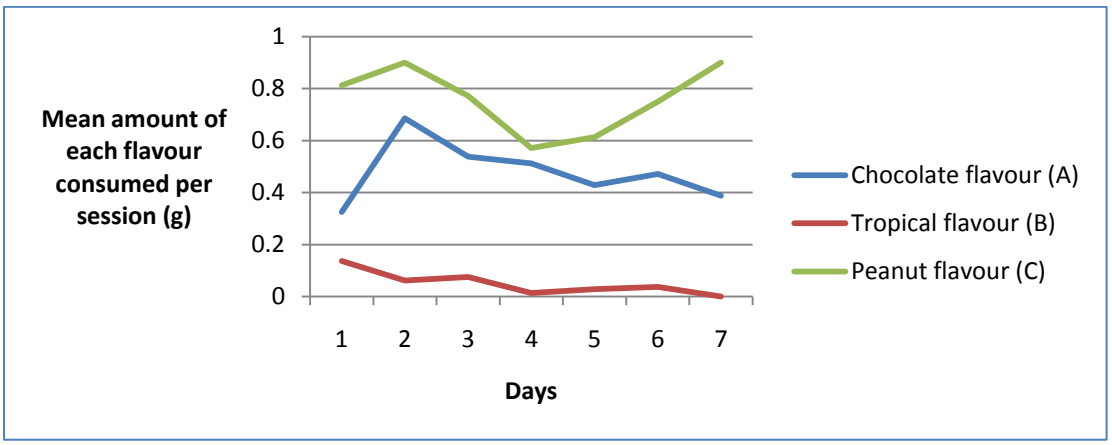
Testing

Each rat experienced each context and its appropriate flavour for five minutes, three times a day for seven days. The decision box did not contain food on the first day of testing due to a problem with the food trays, though this was rectified and this enclosure contained food along with the others from day 2. The animals were rotated though the enclosures in am, noon and pm sessions in such a way as to avoid time of day effects. The animals' behaviour was filmed and the amount of food consumed in each context and in the decision box was recorded each day and averaged across rats as well as across days.

2.9 Results

There is no real habituation effect for any of the context flavours, i.e., the amount of each flavour eaten each day remains relatively stable and there appears to be three clear 'levels of preference', shown by the green, red and blue lines on fig 2.5.

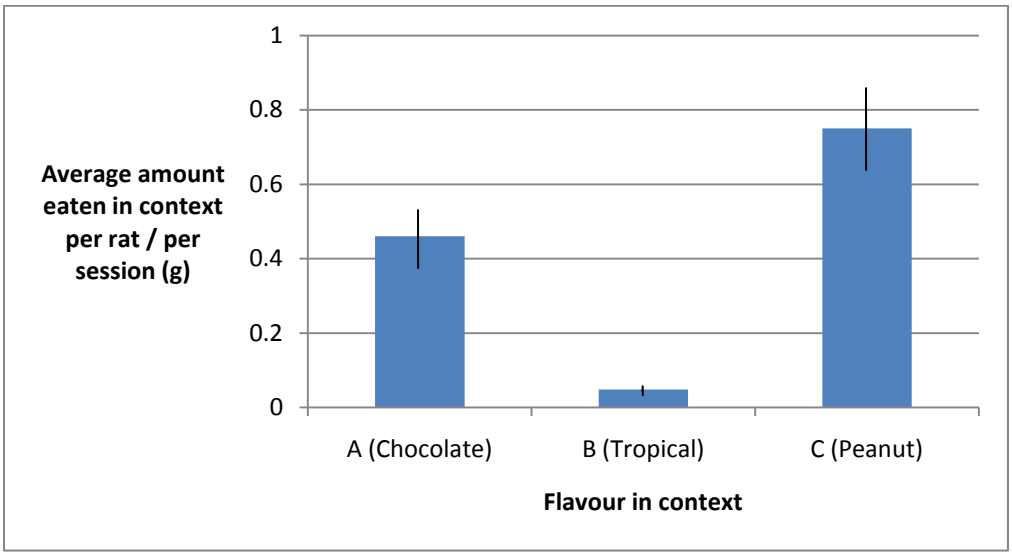
Fig 2.5 Average amount of flavour A, B and C eaten per rat / per session



Assessing A / B / C flavour preference in context

The mean amounts consumed of each of the three flavours were as follows, shown on fig 2.6 below: A (chocolate) = 0.46g, B (tropical) = 0.05g, C (peanut) = 0.74g. This strongly suggests that tropical is the least popular flavour.

Fig 2.6: Mean amount of flavours A, B and C consumed in context, per rat / per session



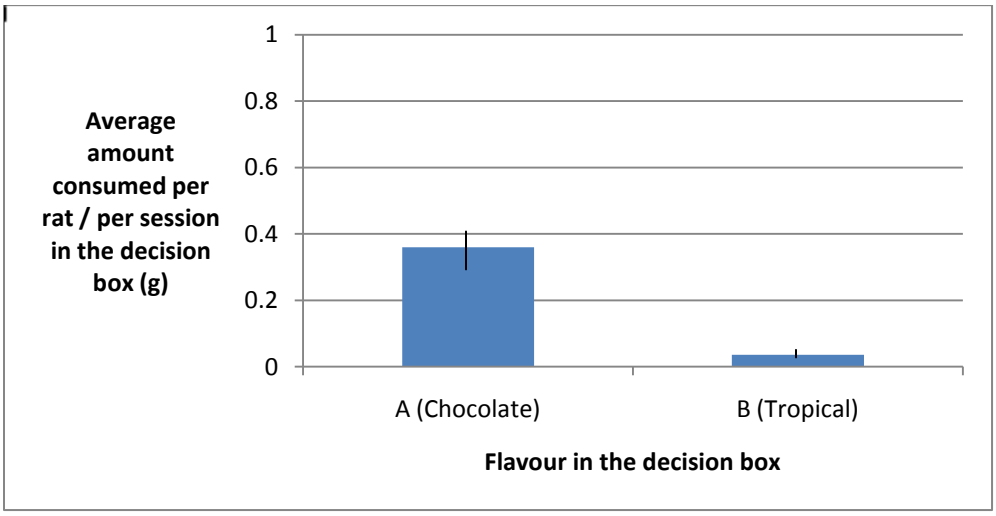
A one-way ANOVA (Repeated Measures Design) conducted on the data showed that there is a main effect here **F (2,18) = 17.643, sig = < 0.05**. Matched-pairs t-tests showed that the mean amount of chocolate consumed (0.46g) was significantly higher than the mean amount of tropical consumed (0.05g): **T_9 = 4.809, sig < 0.05** and also that the mean amount of peanut consumed (0.74g) was significantly higher than the mean amount of tropical consumed: **T_9 = -5.660, sig < 0.05**. There was no significant difference between the mean mounts of chocolate and peanut flavours consumed: **T_9 = -2.030, sig = 0.073.**

Assessing A / B flavour preference in the decision box

It was important to assess the amounts of chocolate and tropical pellets consumed in the decision box as well as in context, to determine if eating behaviour (and preferences) in the presence of a flavour choice differs from that when one flavour is presented in isolation (as in context): Averaged across rats,

the amount of the chocolate choice eaten in the decision box was 0.36g, and the tropical choice 0.036g (means shown below on fig 2.7). A matched-pairs t-test confirmed that there was a significant difference between these two means (**t_9 = 3.788, sig < 0.05**), i.e., when offered a straight choice in the decision box, significantly more chocolate than tropical was consumed.

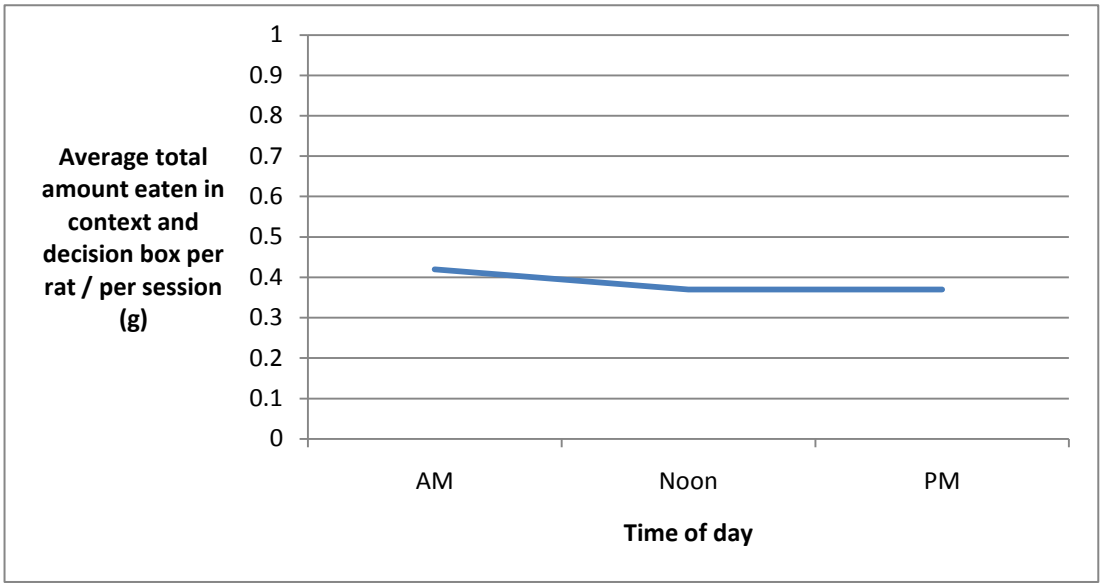
Fig 2.7: Amount of chocolate and tropical eaten per rat / per session in the decision box



Overall eating: time of day effect

Taking all eating in the contexts and in the decision box averaged across rats, slightly more was consumed in the morning session (0.42g) than in the noon (0.37g) or the afternoon (0.37g) sessions, as shown on fig 2.8 below. A one way ANOVA (repeated measures) was used to compare these means, and none of these differences were found to be significant: **F (2,18) = 0.565, sig = 0.578.** At this stage therefore and for the current procedure it can be said that time of day has no bearing on the amount of food consumed.

Fig 2.8: Average amount eaten per rat / per session at different times of day – all flavours in all enclosures



2.10 Discussion

Flavour preference in context

The above results show that the animals ate significantly lower amounts of tropical flavour than either chocolate or peanut. This is particularly problematic as Tropical was selected to be one of the two 'choice' flavours in the decision box, the consumption of which, compared to the consumption of the

other decision box flavour (chocolate), would be used as a later measure of sensory specific satiety and anticipatory sensory specific satiety. Equally, given the very small absolute amounts of tropical flavour consumed in context, it was unlikely that an animal would eat sufficient quantities of this flavour to become satiated by it, meaning it would not be possible to reliably test sensory specific satiety using this flavour.

Flavour preference in decision box

As with eating in context, flavour preference in the decision box was calculated using matched-pairs t-tests (as throughout much of the current study). However, an assumption of this test is that the observations (i.e., the amounts of the two flavours consumed) are independent, and it could be argued here that this is not the case as it is not possible for the animals to eat both flavours simultaneously. This means the consumption of one flavour could be said to affect consumption of the other as (for instance) switching between flavours would take time. It is possible however for the animals to consume the different flavours in the decision box at different rates, which may rectify the issue to some extent (at least in terms of the time taken to switch between them). For studies using similar designs this issue should be taken into account when interpreting the results.

When offered a choice between the chocolate and tropical flavours in the decision box, the animals preferred chocolate, eating a significantly greater amount of this flavour. This is problematic again as it is the decision box flavour choice that will eventually form the critical test element of this investigation and so these two flavours should be as equal in preference as possible. As the tropical flavour appears

to be equally disliked both when offered as part of a choice as well as in context when there is no alternative for the rats to choose, this flavour (B) will be replaced with an alternative flavour B. This alternative will be paired with chocolate in the decision box and stand alone in context B. The following experiment details the introduction of banana as the new flavour B.

Experiment 3 – introducing Banana as Flavour B

2.11 Method

2.11a Apparatus

This remained the same as in the previous experiment, apart from the use of tropical flavoured pellets, which were not used further. Banana pellets, this flavour chosen randomly from the three remaining alternatives, formed the replacement. New food trays were also introduced for the new flavour.

2.11b Design

It was necessary to not only swiftly habituate the rats to the banana flavour itself, but also to the pairing of the banana flavour with context B as well as eating banana in the decision box alongside chocolate. Over this habituation period the animals experienced twice the number of exposures to banana in context B than the other (familiar) flavours in contexts A and C, as well as twice the number of

exposures to the decision box (with the new chocolate-versus-banana pairing) than in the previous run of habituation trials. This experimental avenue lasted three days, considered sufficient to bring the rats 'up to speed' with the new banana flavour.

2.11c Procedure

Each rat experienced five minutes in each one of three contexts, three times a day as before. The animals' experience of the enclosures was rotated such that at each time of day each rat experienced 1) banana in context, 2) the newly-paired decision box and 3) either one of the other two other flavours in its respective context (rotated). The trials were filmed as before and the amounts eaten of each flavour were recorded and averaged per rat / per session.

2.12 Results

Fig 2.10: Average amount of chocolate, banana and peanut flavour eaten per rat / per occasion in context

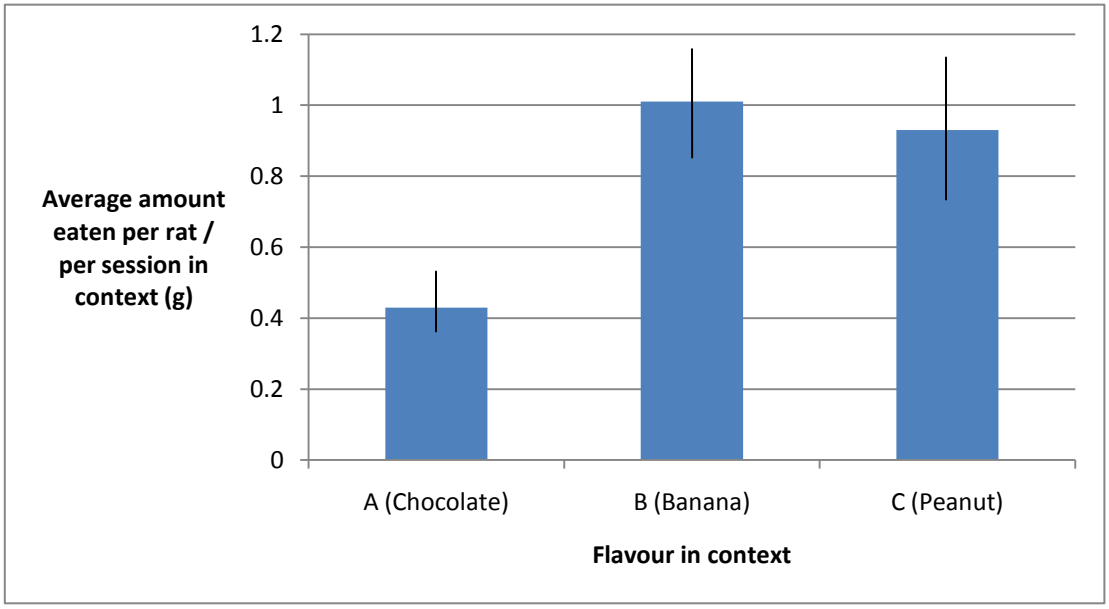
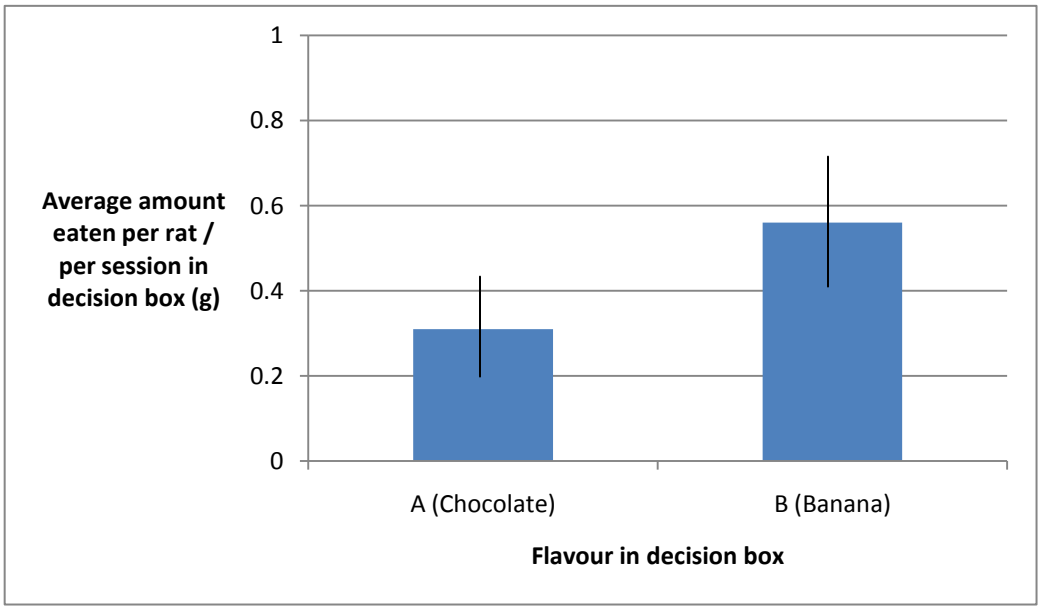


Fig 2.10 shows that in context B banana appears to have been accepted as on a par with the other flavours. The amount of banana eaten in context was 1.01g averaged across rats and exposures – much closer to the favoured peanut (0.93g) than chocolate (0.43g). A one way ANOVA (repeated measures) demonstrated there was a main effect of flavour: **$F_{2,18}$ = 4.689, sig = 0.023**. Matched-pairs t-tests showed that a significantly smaller mean amount of chocolate was eaten than banana (**T_9 = -2.913, sig < 0.05**) and also that a smaller mean amount of chocolate was eaten than peanut (**T_9 = -2.402, sig = 0.040**). There was no significant difference between the mean amounts of banana and peanut consumed (**T_9 = 0.363, sig = 0.725**).

Fig 2.11 below shows that in the decision box, similar *proportions* of chocolate and banana are eaten as in context: chocolate in decision box = 0.31g, banana in decision box = 0.56g. A matched-pairs t-test was carried out and demonstrated that these means (fig 2.11) are not significantly different: **T_9 = -0.978, sig = 0.354, i.e., relatively equivalent amounts of chocolate and banana were consumed in the decision box**. In the decision box it was also observed that on the majority of occasions (25 out of 30) the animals appear to make an initial flavour choice and then exclusively consume that flavour during that session in the decision box, ignoring the alternative.

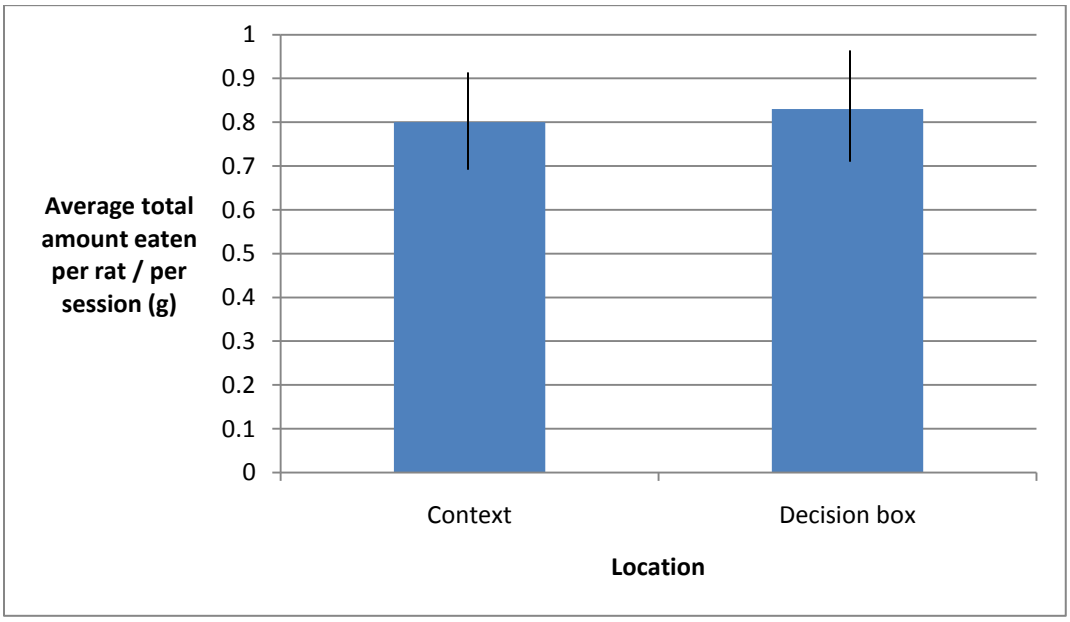
Fig 2.11: Average amount of chocolate and banana eaten per rat / per session in the decision box



Following the establishment of a pair of flavours that are equivalent in terms of palatability (chocolate and banana, above), the overall amounts of food consumed per session in context and per session in the decision box were compared. It would be reasonable to expect that greater proportions would be consumed in the decision box, as the SSS literature (e.g., Johnson & Vickers, 1992; Rolls, 2005; Rolls,

Duijenvoorde & Rowe, 1983) demonstrates that a choice of flavours (colours, textures, etc) to eat results in greater overall consumption. Fig 2.12 below shows the average amounts of food (all flavours) eaten in context and in the decision box (note figure for decision box includes both chocolate and banana).

Fig 2.12: Average total amount of food eaten in context and in the decision box, per rat / per session



A matched-pairs t-test showed that these means were not significantly different:

T_9 = -0.233, sig = 0.821.

2.13 Discussion

Comparing consumption across flavours

In context, the mean amounts of peanut and banana consumed were not significantly different, a positive finding that shows the recently introduced banana flavour was accepted on a par with the already relatively popular peanut flavour. Indeed, the mean amount of banana eaten was significantly higher than that of the more established chocolate (though we can rule out novelty of the new flavour as the driving force due to the aforementioned non significant result when banana was compared to peanut). It could potentially be a cause for concern if it was the case that banana flavour was universally preferred to chocolate as it is these two flavours that are paired in the decision box and would consistently comprise the critical flavour choice when testing SSS in the next chapter. However on comparing the mean amounts of these two flavours eaten whilst in the decision box, there was no significant difference, meaning when these two flavours are presented together, one is no more likely to be consumed than the other. Nevertheless, the preferences of these two flavours will be monitored.

Comparing amounts consumed in context and the decision box

There was no significant difference between the average amount of food consumed per session in context (when a flavour was presented in isolation) and the average amount of food consumed per session in the decision box (when chocolate and banana flavour were presented simultaneously). This is perhaps counterintuitive, as the SSS literature asserts that greater variety results in larger amounts consumed, for all species including humans (Johnson & Vickers, 1992; Rolls, 2005; Rolls, Duijenvoorde &

Rowe, 1983). This is because after an animal has consumed one kind of flavour its relative pleasantness decreases, resulting in a reduced rate of consumption. The opportunity to sample a variety of flavours means this 'flavour fatigue' does not develop to the same extent and therefore the consumption rate should remain relatively high. This phenomenon has been demonstrated with many species and across many sensory qualities, to the extent that humans will consume greater quantities of sweets if there are multiple colours to choose from (Rolls, Rowe & Rolls, 1982). However the eating behaviour of these animals suggests that the choice element in the decision box has no bearing on the amount of food consumed in any one session. It is interesting that on the majority of occasions, the animals consume just one of the two flavours in the decision box and it is therefore possible that the animals are to some extent unaware of the opportunity to sample both flavours, or simply are not inclined to do so. This should not be problematic for the present study as long as the animals are equally inclined to sample either flavour in the decision box, which has shown to be the case.

2.14 Overall Discussion

In line with findings by Smeets & Westerterp-Plantenga (2006), it appears that 5 minutes exposure to a particular flavour is sufficient for satiation to occur for the current group of animals. In the absence of literature providing guidance on how much food may be required to satiate a rat, the present results were taken as a good indication, and it was decided that 2g would be more than sufficient for a rat to produce a state of satiety in one of the current animals. There was also little specific information in the literature with regards to the flavours that rats may find more or less preferable, beyond sweet flavours such as glucose solution being liked and bitter solutions such as quinine being disliked (Berridge & Zajonc, 1991; and others). The present investigation into palatable flavours found that chocolate, banana and peanut flavoured pellets are consumed in sufficient, and sufficiently similar, amounts for these flavours to form the basis of this continuing SSS investigation. Tropical flavoured pellets, when tested here, were almost entirely ignored by the animals, demonstrating a dislike of this particular flavour. Banana and chocolate flavour pellets were paired to form the flavour choice that would be presented later to the animals as part of the test first for sensory specific satiety (SSS) and then for anticipatory SSS. There was no significant difference between the amounts of these two flavours consumed in the decision box, hence this pairing is considered a suitable one. This is an important consideration for any study of SSS, and something Correia et al (2007) tested for also, i.e., it was established that the scrub jays in their study found pine seeds and dog biscuits equally palatable foodstuffs, such that any consumption of one foodstuff over the other at test could be confidently linked to experimental manipulations rather than a base flavour preference. The present results show that all animals are willing to eat either flavour in the decision box, however it is rare for both available flavours to be sampled. This result is unexpected; according to the SSS literature the increased variety in the

decision box should result in both flavours being consumed and therefore a larger amount of food eaten overall, for example Johnson & Vickers (1992) demonstrated that the pleasantness of high variety meals reduces more slowly than the pleasantness of low-variety meals, and Rolls, Rowe & Rolls (1982) established that providing a variety of different coloured sweets induced humans to consume more than if only a single colour was offered. For the purposes of this study however it is sufficient that the animals consume large enough quantities in the decision box and in context to reliably assess flavour preference.

The next step of the study is to establish whether these ten animals demonstrate 'normal' SSS, shown to be a robust phenomenon across many species and sensory characteristics by Rolls and colleagues (1981, 1982, 2005, etc) – i.e., after being satiated by flavour A, do the animals choose alternative flavour B when subsequently offered an A/B flavour choice? The next chapter will investigate this question.

Chapter 3

Establishing SSS

3.1 Introduction

The previous chapter was concerned with investigating how much food is required to satiate rats (2g), the timeframe required (5 minutes) and the flavours of pellet that the animals enjoy to a similar extent (chocolate, banana and peanut). The current chapter is concerned with establishing whether the 10 current animals would demonstrate sensory specific satiety (SSS). This phenomenon refers to the perceived pleasantness of a food decreasing with continued consumption, resulting in other foods being subsequently preferred (Rolls, Rowe & Rolls, 1981). With increasing amounts of a certain flavour or texture etc consumed, the neurons that initially responded vigorously to that particular combination of sensory qualities begin to respond less and less, rendering the food less pleasant to eat (Rolls, 2005). An alternative flavour or texture that is represented by neurons that have not been subject to this reduction in activity is therefore more pleasant to eat.

SSS is a useful tool to utilise for this study due to the robust nature of the phenomenon – there is a large amount of literature demonstrating the proclivity of animals to consume an alternative flavour following extensive exposure to an original flavour. This is shown across many species – humans (Rolls et al 1981), primates (Scott, Yan & Rolls, 1995) and rats (Rolls & Van Duijenvoorde, 1983) as well as across sensory qualities – taste and smell (Duclaux, Feisthauer & Cabanac, 1973), texture (Guinard & Brun 1998) and even the colour of food (Rolls, Rowe & Rolls, 1982) As an instinctive behaviour that promotes natural

variety in the diet it requires no training. It is easy to demonstrate behaviourally and results are quick to achieve and simple to measure.

However what is not clear, and the focus of this study, is whether rats can anticipate satiation to a particular flavour. If this is the case an animal offered a choice between two flavours while expecting satiation to one of them in the near future, would find the alternative more pleasant to eat (Raby, Alexis, Dickinson & Clayton, 2007). An animal showing this behaviour would demonstrate an ability similar to that described as 'future planning' or 'episodic future thinking' in humans, i.e., acting in response to a future motivational state rather than one currently being experienced (Atance & O'Neil, 2005). Experiments with scrub jays have demonstrated that this species appears capable of this kind of satiation anticipation (Correia, Dickinson & Clayton, 2007), such that these birds will *ignore* current SSS-based drives and choose to cache a type of food to which they have just been exposed for 3 hours, because they are aware that just prior to *retrieving* their caches they will be presented with an alternative flavour, rendering the cached flavour more palatable than it is at present.

This study ultimately seeks to establish whether rats will alter their eating behaviour in such a way that makes it obvious that they are making provision for a future occasion in which their needs will be different from those currently being experienced, i.e., by choosing to eat the flavour that is different to that which they will soon be satiated on. However it is first necessary to establish that the current group of animals are able to display 'normal' SSS behaviour prior to investigating whether or not the animals will show anticipatory SSS (see next chapter).

The rats will be exposed to satiating amounts of a certain flavour in the appropriate context (chocolate, banana or peanut) for 5 minutes, and then the rats will be removed from the context and placed in a neutral decision box and presented with a flavour choice, which will always be chocolate versus banana. If the animals demonstrate SSS, after eating chocolate in context, an animal will eat a greater proportion of banana (in this case the incongruous flavour) than chocolate (the congruous flavour) in the decision box, and vice versa. Following consumption of peanut flavour in context, the animals will have no reason to prefer either chocolate or banana flavour in the decision box, and therefore may be expected to sample both flavours, behaviour shown by Correia, Dickinson & Clayton's (2007) jays, demonstrating a reduced drive towards either one of the flavours.

3.2Method

Experiment 1: testing SSS

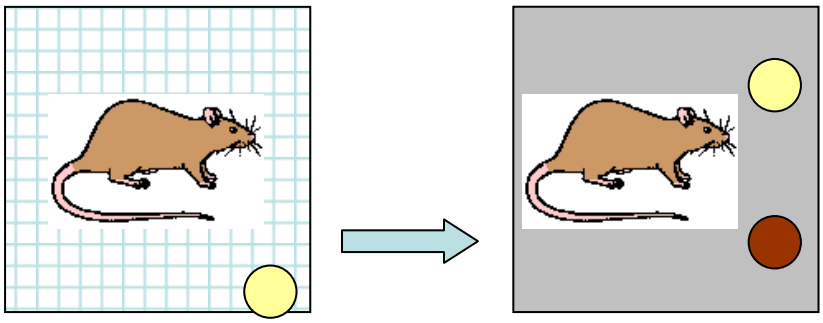
The subjects and apparatus remained identical to the previous chapter

3.2a Design

The test animals experienced the following sequence (see fig 3.1)

1. placed in context A, B or C, with 2g of the appropriate flavour (5 min)
2. moved to decision box, containing an chocolate vs. banana flavour choice (5 min)

Fig 3.1: sequence of movements to test sensory specific satiety



5 min in context with 2g appropriate flavour

5 min in neutral decision box with flavour choice:

Chocolate versus banana

This occurred twice a day, first in the morning at approximately 9am then again at 3pm. The time delay between sessions and others later in the study was at least three hours. Experiments by Hetherington, Rolls & Burely (1989) demonstrated that the pleasantness of a satiated food was to a large extent restored an hour after satiation, therefore this six hour delay was considered more than sufficient to ensure earlier testing sessions did not interfere with later ones.

Throughout the testing days the rats experienced contexts in the consistent order: A followed by B followed by C. In the decision box the left/right positioning of the A/B trays in the morning and afternoon was counterbalanced. This meant that after 6 days each rat had experienced 12 runs altogether and so four in each context, meaning two in the morning and two in the afternoon, and one of these with A on the outside of the decision box, and one with B on the outside.

3.2b Procedure

A rat was placed in context, with the appropriate flavour already present, as the timer and video recorder were started. The rat was filmed for five minutes, then the recorder was stopped and the tray of food was removed and weighed to establish the amount of food eaten. The rat remained in context while the food was weighed and the amount recorded, then the rat was picked up and placed in the decision box with the A and B food trays already present. The timer and recorder were started once again, and after five minutes these were both stopped and the rat removed from the decision box and returned to its home cage. The amounts of flavours A and B consumed were recorded. When possible

more than one rat tested simultaneously, by running in more than one context at a time, and staggering the start times such that only one rat needed to be in the decision box at any one time.

If an animal ate flavour A in context and then continues to eat flavour A when transferred to the decision box, this animal would have eaten *congruously* (i.e., ate the same flavour). An animal that ate flavour A in context followed by flavour B in the decision box would be said to have eaten *incongruously* (i.e., ate the alternative flavour). It is this incongruous eating behaviour that would demonstrate SSS.

The data were processed by calculating a simple ratio comparing how much of the incongruous flavour was consumed as compared to the congruous flavour, giving an 'incongruity score'.

The incongruity score was calculated as described below, such that a score of +1 would mean that all animals ate entirely incongruously, i.e., that after eating A in context all animals ate nothing but B in the decision box, and vice versa. A score of -1 would show the opposite, and a score of zero would demonstrate that on average the animals showed no flavour preference in the decision box whatsoever. The overall group mean score is then compared to zero via a single sample t-test, to establish whether it is statistically different from the zero 'chance' value.

The calculation to establish the average incongruity score is set out below; for each animal, the average amount of congruous flavour eaten in the decision box was subtracted from the average amount of incongruous flavour eaten in the decision box, and the result was divided by the total amount of food eaten in the decision box. Dividing the amounts of each flavour eaten by the total amount eaten may help to control for individual animals consuming different amounts of food, as well as providing an easy to read score for each individual animal:

$$\frac{\text{(Incongruous eating in decision box)} - \text{(congruous eating in decision box)}}{\text{(Total amount eaten)}}$$

For example, after exposure to context A, a rat eating entirely incongruously would only consume flavour B subsequently in the decision box – for example maybe this animal eats 0.5g of B.

Therefore incongruous minus congruous eating here gives 0.5 – 0 = 0.5.

Dividing this resulting 0.5 by the total amount eaten (0.5g) would give 0.5/0.5 = 1.

This demonstrates that entirely incongruous eating produces a maximum *incongruous eating score* of 1 which would show perfect SSS. Conversely if an animal eats entirely congruously (consumes only A in the decision box following A in context for example) this would result in a minimum score of -1. As aforementioned, a score of zero means that an animal eats equal amounts of A and B in the decision box regardless of the flavour previously experienced.

This calculation then is a measure of an animal's preference for the incongruous flavour over the congruous or vice versa, and means that this one score takes into account the amount of A and B eaten in the decision box following exposure to A in context, and the amount of A and B eaten in the decision box following exposure to B in context. A more positive score shows the incongruous flavour is preferred over the congruous (A is preferred after B, and B is preferred after A) and a more negative score the opposite (A is preferred after A and B is preferred after B). Also as aforementioned, the score calculated is compared statistically to that score which would be achieved by chance – i.e., a score of zero resulting from the animals eating equal quantities of A and B in the decision box as if the animals had no preference. This will determine if any apparent preference for one flavour over the other can be accepted as statistically significant.

In addition to this calculation, it was decided to test once again for time of day effects to determine whether eating in the morning or the afternoon might have a bearing on the amount of food consumed. It was considered appropriate to investigate time of day effects at this particular stage because the

procedure as it currently stands is now very similar to the procedures to be used for the remainder of the study, in terms of the apparatus used and the pattern of flavour-exposure. Therefore any time of day effect at this stage could be expected to reoccur at future points and would need to be taken into account when designing the future planning test.

3.3 Results

First it was determined whether the rats demonstrated incongruous eating (SSS), as explained above, and the incongruity score for each rat and the average for the group is shown below in table 3.1.

Incongruity scores

Table 3.1: Incongruity scores for each rat, and averaged.

Rat	Incongruous eating score
1	-0.2
2	0.076
3	0
4	0.98
5	0.021
6	0.62

7	0.33
8	-0.016
9	-0.51
10	0.92
Average	0.22

As aforementioned, a score of zero demonstrates that equal amounts of the congruous and incongruous flavours were eaten in the decision box regardless of the flavour previously experienced in context (animal three demonstrates this perfect zero score above). A score above zero demonstrates that more of the incongruous flavour is eaten relative to the congruous flavour. This means that if an animal is exposed to context A (and therefore accompanying flavour A) and then placed in the decision box with an A/B flavour choice, more B than A would be consumed here. A score of 1 demonstrates that only the incongruous flavour is consumed in the decision box, i.e., that in the above scenario a rat eats *only* flavour B. This would suggest that an animal had been successfully satiated by flavour A in context, resulting in this flavour seeming subsequently less pleasant in the decision box, resulting in flavour B being consumed exclusively.

On average the animals present an incongruous eating score of 0.22. To assess whether or not this demonstrates genuine tendency towards SSS it is necessary to establish whether this mean score is

significantly different to the mean incongruity score which would be expected by chance, i.e., if there was no flavour preference in context, i.e., a score of zero. A single sample t-test was carried out, comparing each score to the theoretical zero score as explained above: **t_9 = -1.449, sig = 0.181** demonstrating that there is no significant difference between the score obtained and the chance score of zero.

This result may be influenced by flavour preference; it was observed in the previous chapter that, following the introduction of banana flavoured pellets, this flavour was consumed significantly more than chocolate by the animals when in context. At that stage there was no significant difference between the consumption of those two flavours in the decision box so it was appropriate to continue with this pairing. However it was now important to revisit the possibility of a flavour preference in order to determine the reason for these animals not showing SSS here. As aforementioned, the incongruity score calculated above expressed the relative amount of incongruous eating in the decision box following exposure to context A and context B. In order to investigate flavour preference further, separate incongruity scores were calculated for decision box eating following exposure to contexts A and B:

Table 3.2: Incongruity scores for each rat following exposure to context A and context B

Rat	Incongruous eating score following context A	Incongruous eating score following context B
1	0.60	-1.00
2	-0.18	0.33
3	1.00	-1.00
4	0.96	1.00
5	0.88	-0.84
6	1.00	0.24
7	1.00	-0.33
8	0.97	-1.00
9	-0.90	-0.11
10	0.83	1.00
Average	**0.62**	**-0.17**

As shown in table 3.2, the average incongruity score for the group of animals is considerably higher following exposure to context A (0.62) than exposure to context B (-0.17). As before, these average scores were compared using single sample t-tests to the theoretical zero scores which would occur for each animal if congruous and incongruous eating was the same. For the post-A congruity score: **T_9 = -3.024, sig <0.05** and for the post-B incongruity score: **T_9 = 0.679, sig = 0.514.** These results show that the higher mean score following context A is significantly different from zero and means that after exposure to context A the animals consume more of flavour B relative to flavour A. However the score following exposure to flavour B in context is not significantly different from zero – i.e., after exposure to context B an animal is no more likely to consume incongruous flavour A than congruous flavour B. This means that SSS occurred following exposure to flavour A but not B, which points to a flavour B preference.

As planned, the average amounts of chocolate and banana consumed in context and in the decision box were examined. Fig 3.2 below shows the average amount of chocolate and banana consumed in the decision box. As the aim was to establish a general flavour preference unrelated to the context previously experienced, all decision box eating was included in the calculation including that which occurred after exposure to context (and therefore flavour) C.

Fig 3.2: The average amount of chocolate (A) and banana (B) eaten per rat / per session in the decision box (*including following flavour C in context*)

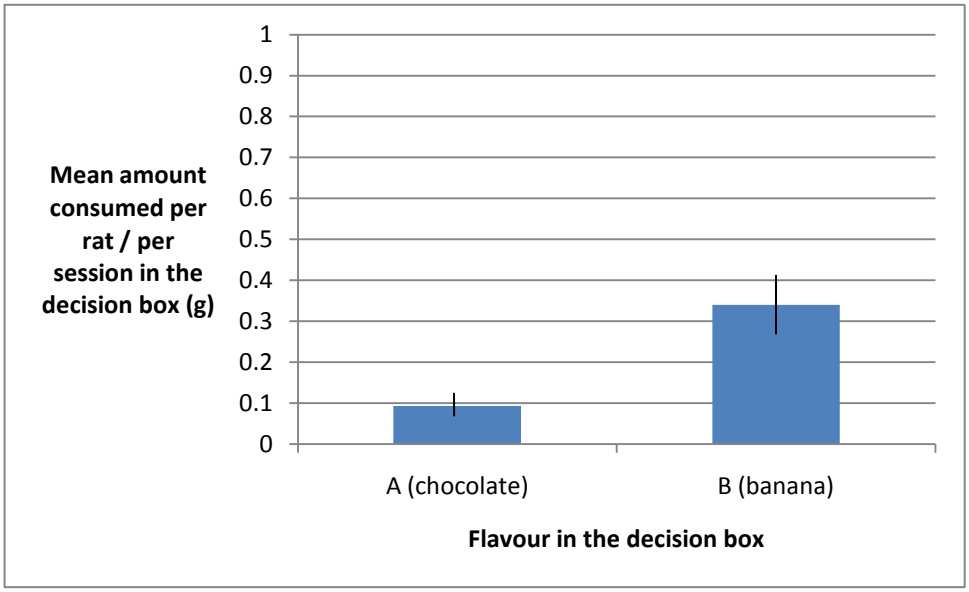
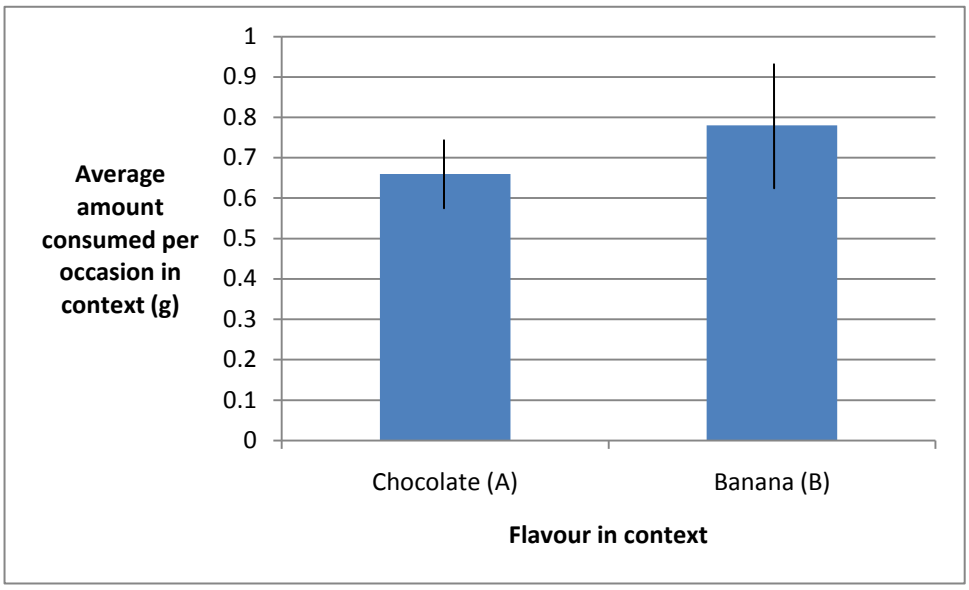


Fig 3.2 shows that when comparing average amounts of chocolate and banana eaten across all occasions in the decision box, a higher mean amount of banana (0.34g) than chocolate (0.093g) was consumed – and in fact all but two of the animals ate greater amounts of banana than chocolate flavour. A matched-pairs t-test showed that this difference was significant: **T_9 = -2.291 sig < 0.05.**

As planned, the amounts of chocolate and banana eaten in context were also examined, in order to determine whether the banana preference established above was just a feature of decision box eating (i.e., involving a direct flavour choice), or whether greater amounts of banana than chocolate were also consumed in context when these flavour was experienced in isolation. Fig 3.4 below shows the average amounts of chocolate and banana consumed in context throughout the testing period. A matched-pairs t-test on the data demonstrated there was no significant difference between the mean amounts of A

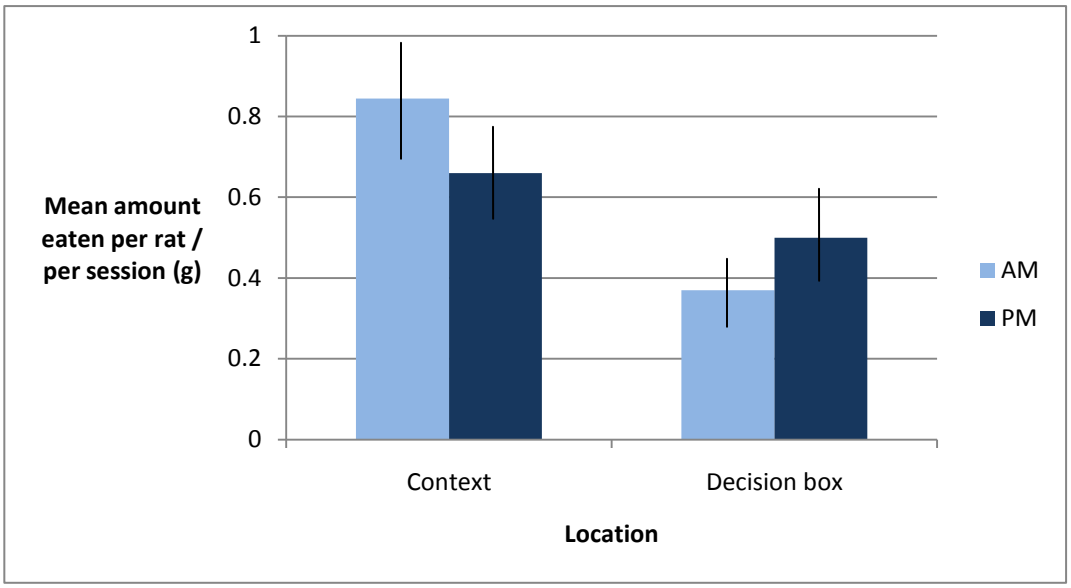
(0.66g) or B (0.78g) eaten in context: **t_9= -1.359, sig = 0.207.** It would appear then that both flavours are equivalently palatable in isolation, however when offered a choice nearly all animals would prefer banana.

Fig 3.4: The average amounts eaten of flavours A and B in context, per rat / per session



As aforementioned, it was also considered important to assess any impact of a time of day effect on amount of food eaten. The mean amount of food consumed per session in context (all 3 flavours combined) and the mean amount consumed in the decision box (both flavours A and B) was calculated, shown on fig 3.5 below.

Fig 3.5: Average amount of food consumed per rat / per session in the morning and in the afternoon, for both eating in context and eating in the decision box:



A repeated-measures two-way ANOVA was carried out on the data shown in fig 3.5 above to establish if the mean amount of food consumed was affected by time of day, location of consumption, or an interaction between the two. The results demonstrated that there was no effect of time of day (equivalent amounts of food were eaten overall in the morning and the afternoon): **$F_{1,9}$ = 0.356, sig = 0.566** and no overall effect of location: **$F_{1,9}$ = 4.005, sig = 0.076**. However there was an effect of the interaction between location and time of day, with time of day having differing effects in context and the decision box: **$F_{1,9}$ = 8.072, sig = <0.05.** Matched-pairs t-tests were carried out to further investigate this.

In the mornings, significantly greater amounts were consumed in context (0.84g) than in the decision box (0.37g): **t_9= 2.742, sig = <0.05.** However in the afternoons there was no such difference in context

(0.66g) and decision box (0.50g) eating: **t_9= 1.015, sig = 0.337.** In addition, greater amounts were eaten in context in the morning (0.84g) than were eaten in context in the afternoon (0.66g): **t_9= 2.934, sig = <0.05.** However there was no difference in the amount eaten in the decision box in the morning (0.37g) and afternoon (0.50g): **t_9= -0.520, sig = 0.163.**

Taken together, these results show it is not the case that the animals eat most 'in the morning' or that the animals eat most when in context, but they do eat more when in context in the morning, i.e., the animals eat more of the first experimental food to which they are exposed on any given day. Therefore in the following chapter, as the first food to be experienced in any **critical testing run** will be the food choice in the decision box, it will be preferable to carry out *critical tests* for future planning in the morning, to take advantage of this initial eagerness to consume the first food experienced.

3.4 Discussion

The average incongruity score (0.22) is not significantly different from that score that would be obtained by chance, i.e., if the rats showed no flavour preference whatsoever (a score of zero). This means that the animals do not consume proportionally more of the incongruous than the congruous flavour when presented with a flavour choice in the decision box, therefore the above experiment does not

demonstrate that this group of animals show SSS. If decision box eating following flavour A and B in context is separated out, it can be seen that the eating behaviour following each of these two flavours is different. Following flavour A in context the animals consume significantly greater absolute amounts of flavour B than A in the decision box. However following flavour B in context there is no significant difference between the absolute amounts of A and B eaten in the decision box. Therefore SSS could be said to occur following flavour A in context (more B subsequently eaten), but not following flavour B in context (equal amounts subsequently eaten). This would suggest that there is a preference for flavour B in the decision box which facilitates this result for SSS following flavour A. Subsequent analysis to investigate this showed that in the decision box, the average amount of B consumed per occasion is significantly higher than the respective amount of A consumed. Therefore in the decision box there is a significant preference on average for flavour B over A.

However there is no such flavour preference when considering flavours A and B in context – i.e., when presented with the flavours in isolation there is no significant difference in the amount of each flavour consumed. This means it can be assumed that regardless of the context to which the animals are exposed prior to the decision box, the amounts of food consumed therein will have been relatively similar. Despite the flavours appearing equally palatable in isolation, if given a direct choice between the two flavours in the decision box the animals on average do prefer flavour B.

This presents a problem as it is the amounts eaten in the decision box that will form the basis of conclusions regarding the animals' tendencies towards SSS and subsequently their future planning abilities. It is therefore important that the two flavours offered in the decision box are equivalently palatable to the extent that the tendency to eat either one of them can be altered by the flavour

previously experienced by an animal (i.e., more A is eaten after B and vice versa). When the same flavour choice is later used to test future planning, it is important that the animals' choice is affected only by their anticipation of upcoming satiation, rather than a current intrinsic preference for one flavour more than the other.

For this reason it was considered important for the decision box flavours to be changed such that A and B would no longer be paired. B and C would be tested to establish if these flavours could form a more equally weighted decision box. If successful, the animals should demonstrate SSS when subsequently tested under the new B vs. C decision box circumstances.

As the rats still consumed flavour A in context it was not necessary to remove the flavour from the study, as had been required previously with the tropical flavoured pellets. Therefore context A remained associated with chocolate (which remained flavour A), but the decision box would now feature flavour B (banana) and flavour C (peanut). The following experiment describes the swapping process and assesses its success.

Experiment 2: swapping A for C in the decision box

3.5 Method

The subjects and apparatus remained the same as before

3.5a Design

To successfully habituate the animals to a decision box containing flavours B and C, they were exposed exclusively to this environment for several days. It was not considered necessary to intersperse this with experience of the flavours in context, as this aspect of the design remained entirely unchanged.

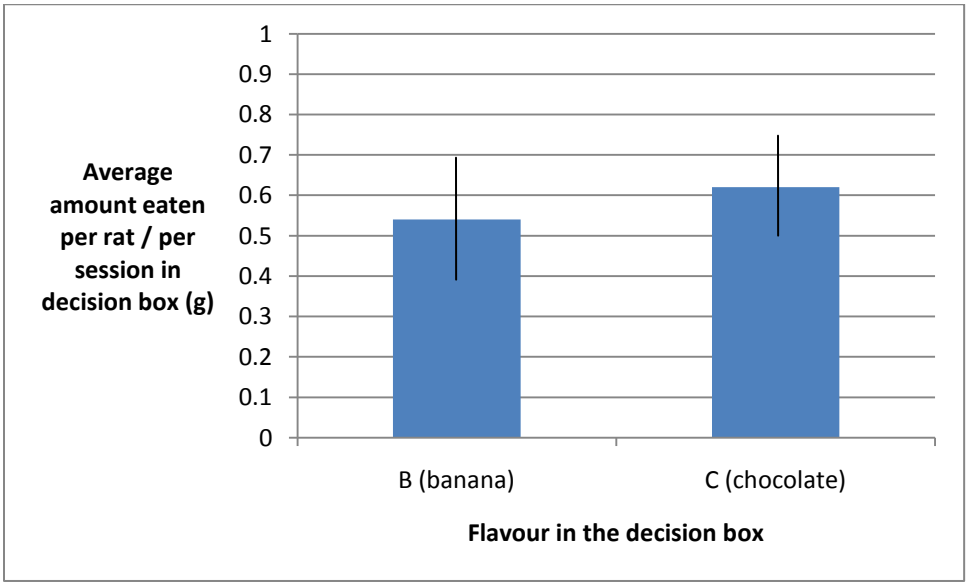
3.5b Procedure

Each rat experienced the 'new' decision box three times a day (at approximately 9am, 12 noon and 3pm) for two days, for five minutes each time (60 runs in total). The food trays containing pellets were present whenever a rat was placed into the decision box. Each run of was recorded as before using the

same video recorder, and after each run the amounts of B and C consumed were weighed and recorded, and the trays replenished before the next rat placed in the decision box. The banana and peanut trays were left/right alternated such that each rat experienced both banana and peanut on the left and right in the morning, at noon and in the afternoon an equal number of times. This allowed for variation in motivation or hunger levels at different times of day as well as controlling for any possible preference for one side of the box or the other.

3.6 Results

Fig 3.6: following 6 runs of B vs C in the decision box, per rat / per session



As shown by fig 3.6, Flavours B and C appear equivalently palatable, with the mean amount of each flavour eaten over the habituation period calculated as banana = 0.54g and peanut = 0.62g respectively. A matched-pairs t-test demonstrated that there is no significant difference between these means: **t_9 = -0.330, sig = 0.749.**

Additionally there were no consistent individual flavour preferences, with all animals frequently switching between both flavours between occasions. Within each session, in all but 2 of the 60 runs only one of the two flavours was consumed, i.e., it was very rare that both flavours were sampled on any one occasion, despite the opportunity to do so.

3.7 Discussion

The average amounts of B and C eaten in the decision box were not significantly different, and therefore it can be taken that these flavours are sufficiently similar in their palatability for SSS to be confidently re-tested using this more balanced version of the decision box. It can now be assumed that the animals will not consistently choose either one of the flavours over the other, meaning a greater confidence can be placed in the results.

It is notable that during any particular session in the decision box an animal only very rarely samples both of the flavours, instead tending to select and consume one of either B *or* C. This suggests that the animals are certain of the flavour choice they are making, as it is very rare for the alternative flavour to be sampled after an initial choice is made.

The improved B vs. C flavour pairing in the decision box now allowed for a repeat of this chapter's first experiment in an attempt to demonstrate the presence of SSS in these animals.

Experiment 3: re-testing SSS

3.8 Method

The subjects, apparatus, design and procedure were identical to experiment 1 of the current chapter, apart from the altered decision box flavours – now B and C in place of A and B. The experiment ran for a further 6 days, twice a day, as before. The results were processed in the same way as the first experiment, using incongruity scores to assess the extent to which the animals consumed the flavour to which they had not just been exposed in context. Due to the altered decision box flavours, the focus on flavours in context also changed in order to maintain congruity and incongruity – the results were considered in terms of how much B and C were eaten in the decision after being exposed to flavours B and C in context, rather than after flavours A and B in context, as in the previous experiment.

Also, it was decided that if an animal consumed nothing in context, any subsequent flavour choice and consumption in the decision box should be excluded from the analysis, as it was considered that any such decision box choice could not have been the result of experiencing a previous flavour.

3.9 Results

The incongruity scores were calculated as before, using the incongruous amount eaten in the decision box minus the congruous amount, divided by the total amount consumed in the decision box. As before, the positive scores show more incongruous than congruous eating, and hence a tendency towards SSS. Zero demonstrates no preference, i.e., equal amounts of incongruous and congruous flavours consumed.

One animal (rat 7) was excluded from the current analysis due to this animal consistently consuming nothing in context, meaning that anything consumed subsequently in the decision box could not have been the result of satiation to the context's flavour. The results for the remaining nine animals are shown below in table 3.6.

Table 3.6: Incongruity scores for each rat showing the extent of each animal's incongruous eating

Rat	Incongruity score
1	0.63
2	0.44
3	1.00
4	0.30
5	0.11
6	0.56
8	0.50
9	0.24
10	0.56
Average	**0.50**

As shown by the above table, on average the group have an incongruity score of 0.50. This is significantly greater than the average score that would be obtained by chance (zero) if all animals were impervious to the different flavours and ate equal amounts of each and obtained a score of zero (matched-pairs t-test): **t_8= -5.597, sig < 0.05**. It is also possible, due to this calculation, to assess the individual scores of each animal – as can be seen from the above table, every animal has a positive

incongruity score (as opposed to when chocolate and banana were paired previously, which resulted in several animals with negative scores) and 6 of the 9 animals have a score of near or above the significant average of 0.5.

3.10 Discussion

Following the change to a decision box offering a B versus C flavour choice there is evidence for the group showing above chance incongruity scores. This means that following exposure to flavour B or C in context, the animals show a tendency to eat a significantly larger proportion of the incongruous flavour when subsequently placed in the decision box with a B/C flavour choice. For example, an animal exposed to flavour B in context then eats a greater proportion of flavour C when subsequently placed in the decision box, and vice versa, i.e. the current group of animals demonstrate SSS, in line with previous studies that have demonstrated this ability in rats (Rolls et al 1983) This final, significant result for SSS was the result of altering the flavour choices in the decision box such that both flavours were similarly palatable. This ensured that the animals lacked a B/C preference and so the choice made in the decision box could be put down to an animal's previous experience of a particular flavour in context. This result also confirmed that these animals can discriminate between the decision box flavours as they choose one preferentially over the other.

As a result, the present study can now investigate anticipatory SSS. This will require the animals to demonstrate the same significant preference for the incongruous flavour choice in the decision box, but *prior* to any exposure to a satiating flavour.

Choosing the alternative flavour prior to the satiation is a behaviour that only makes sense if the animal is anticipating the upcoming satiation, as both flavours are equally palatable and therefore there is no reason for one flavour to be preferred over the other, *unless* the future experience is taken into account. To ensure that this remains the case it is important that the animals anticipate the upcoming satiation yet *do not* anticipate the flavour choice they offered prior to it. Several studies of both memory (Schwartz et al 2005) and future planning (Emery et al 2001) in nonhumans have allowed the possibility that crucial decisions were made in advance and stored as semantic knowledge, then utilised as such when the animal is presented with a choice. This does not allow for a spontaneous 'on the spot' decision because semantic rather than flexible episodic mechanisms are being utilised (Zentall 2005). In this study an advanced choice would also present a practical problem: An important aspect of the methodology is that any flavour choice is offered in a neutral environment (the decision box) as opposed to in a context, which ensures that flavour decisions are not influenced by the surrounding environment. An animal expecting an upcoming choice may make the decision in advance, before being placed into the neutral decision box and therefore while still in context. As every context is associated with a certain flavour this introduces the possibility that a flavour choice would be made based on that present association rather than genuine anticipation of a future context or flavour. It has been demonstrated that an association between a specific location and a specific food increases consumption of that food in that location (Petrovich, Ross, Gallagher & Holland, 2007).

Therefore it is necessary to train the animals to anticipate exposure to a certain flavour, but surprise them with an unexpected flavour choice before they are exposed to this anticipated flavour. This means any decision made in the decision box will have been made on the spur of the moment and influenced only by the animal's anticipation of the upcoming satiating flavour.

If under these circumstances the rats can demonstrate the ability to choose the 'incongruous' flavour as they did above, it would be in anticipation of satiation which has not yet occurred. They would therefore be showing ability akin to future planning. **The rats would be acting presently, but motivated only by the anticipation of a future state rather than any state currently being experienced.**

Chapter 4

Establishing anticipatory SSS

4.1 Introduction

In the previous chapter it was successfully demonstrated that the current group of animals display eating behaviour in line with SSS (Rolls, 2005). This means that these animals eat to satiation when exposed to 2g of flavoured pellets for five minutes, and then when subsequently offered a choice between this same flavour and an alternative, they consume a significantly greater proportion of the alternative flavour. This reduction in pleasantness with continued consumption of any foodstuff is a robust phenomenon that aids in the consumption of a varied diet – to the extent that an excessively varied diet contributes to obesity in rats, as demonstrated by Rolls, Duijenvoorde & Rowe (1983). The current chapter aims to establish whether the current animals can demonstrate *anticipatory* SSS, i.e., whether they can be trained to expect exposure to a certain context and hence anticipate satiation by the context's associated flavour. If this is the case, when offered a flavour choice *prior* to this expected satiation, the animals should consume more of the flavour that they are not expecting (the incongruous flavour), ensuring that the pleasantness of the anticipated flavour remains undiminished in preparation for the animal's exposure to it. A similar method has been utilised to test for future planning ability in scrub jays – Correia, Dickinson & Clayton (2007) demonstrated that jays would choose to go against their natural urges and cache a foodstuff to which they were entirely satiated, in preparation for retrieving the cache, which would occur after satiation by a *different* foodstuff (hence the original cached food would be renewed in pleasantness). An important aspect of this study was that it was not a

repeated procedure, such that the birds could not 'learn' from many trial-and-error experiences which food would be most rewarding to cache. After several training trials, there was only 1 testing trial, demonstrating a spontaneous ability to consider a future state of satiety. This spontaneity is equivalent to one of the criteria put forward by Griffiths, Dickinson & Clayton (1999) for the testing of nonhuman episodic memory. It was considered that this spontaneity (in response to a 'one off' test) was required in order to show that an animal was genuinely recalling an episode, rather than simply displaying 'knowledge' of a repeated procedure. The same criterion applies for envisaging future occasions (Correia et al, 2007). An important aspect of the present study is that the flavour choice offered to the animals is infrequent, rendering the choice unexpected and requiring a spontaneous response based on genuine anticipation. Zentall (2006) particularly emphasised the importance of an unexpected choice when investigating nonhuman mental time travel, be is retrospective or prospective. He cites the example of asking someone what they had for breakfast. If this question was unexpected, a person would have to actively recall what it was they had eaten. If that person had been asked the same question for many days then the memory of breakfast may instead be stored as semantic knowledge – i.e., they just 'know' they had eggs on toast, and no active recall of the actual event is necessary. This applies to nonhuman studies as well, in that an animal's expectation of an upcoming choice or test introduces the possibility that any response is not based on genuine recall or forward planning, but semantic 'knowledge' of what the right answer is. As long as the flavour choice in the present study is unexpected, an incongruous flavour choice would demonstrate an ability to act in a way that serves the animals' future rather than present needs, i.e., by choosing to consume more of the flavour that is *not* going to be consumed in the near future, thus helping to maintain the pleasantness of the upcoming flavour and therefore ensuring that consuming this flavour is rewarding. There would be no reason for the rats to choose this incongruous flavour unless engaging in some kind of planning ability that acknowledged the flavour to which they would soon be exposed; this is because the flavours used in the

study are equivalently palatable, and it has been established already that the animals do not hold individual flavour preferences. Therefore a successful demonstration here of anticipatory SSS would provide strong evidence that these animals are making a choice in the present that only makes logical sense if a future situation is being considered.

The animals therefore first had to be trained to anticipate a certain flavour (training phase). The simplest way to achieve this was to teach the animals to expect exposure to a certain context. The contexts had been paired with certain flavours since the earliest days of the study and therefore anticipation of a context could also be taken as anticipation of the context's associated flavour.

The animals were trained to anticipate a context by repeatedly exposing them to an identical sequence of contexts, such that after a high number of repetitions the animals will come to anticipate the next step in the sequence. It is at this point, when the animal is anticipating a subsequent context (and associated flavour), that the prior-choice would be presented (the test phase).

No study has yet demonstrated this forward planning ability in rats, or in any species other than Correia, Dickinson & Clayton's (2007) scrub jays, a species which has a natural proclivity to cache food with the intention of later retrieving it. This instinctive tendency may aid the birds' ability to make such decisions with the future in mind. A demonstration of this ability in a different species would be hugely important with respect to developing animal models of the kinds of human conditions that result in the degeneration of these abilities of memory and future planning, such as Amnesia (Rosenbaum, Koler, Schacter, Moscovitch, Westmacott, Black, Gao, Tulving, 2005; Hassabis, Kumaran, Vann & Maguire,

2007) and Alzheimer's disease (Collie & Maruff, 2000; McKhann, Drachman, Folstein, Katzman, Price & Stadlan, 1984).

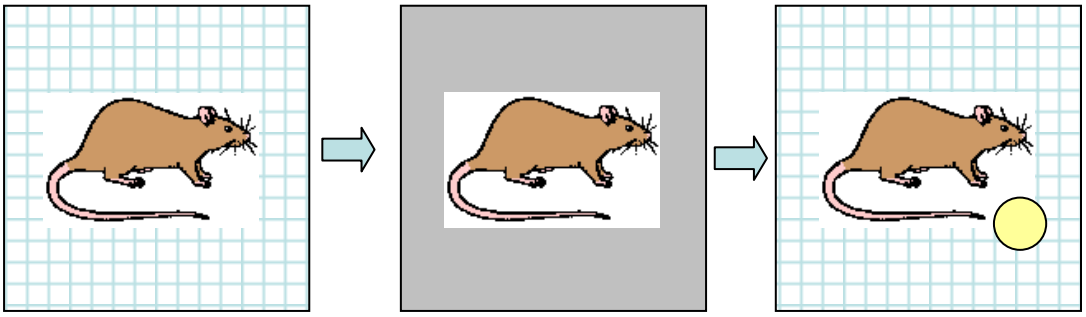
4.2 Method - training

As aforementioned, this part of the study is split into 1) training and 2) test phases. The subjects and apparatus remained as in previous chapters.

4.2a TRAINING design

The training phase involved moving the animals between the contexts and decision box in a consistent order, such that the animals would become accustomed to this order and come to expect it. There were two kinds of training sequence through the animals were consistently moved, 1) Context runs - featuring either context A, B or C - or 2) Decision Box runs. Context runs involved a particular context and the decision box, but Decision Box runs involve the decision box *only*.

A Context run occurred as below:



5 min in empty context 'X'

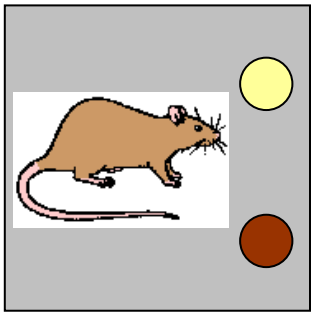
5 min in empty decision box

Returned to same context 'X' with appropriate flavour for 5 min

A rat is placed into an empty context for 5 minutes. The rat is then transferred to the empty decision box for 5 minutes. Meanwhile the appropriately flavoured food is added to the original context, such that when the animal is returned to the context, the associated flavour is present. After 5 minutes exposure to the context and flavour the animal has completed its run and is returned to its home cage. The above sequence comprised one 'run'. If a run featured context A this was an 'A run', context B a 'B run' etc. The context that an animal was initially exposed to was always the context to which the animal was returned after the decision box. Training the animals to this sequence served a number of purposes:

1. The animal learned that the context to which it was first exposed was the same one to which it would be returned later, hence laying the groundwork for anticipating the return to any particular context
2. The animal learned to expect food (and of the appropriate flavour) on its *return* to the context
3. The animal became accustomed to being in the decision box immediately before its return to the anticipated context and flavour. This was important as during the test phase it would be while in the decision box that the animal is presented with the prior-choice. The animal must therefore feel comfortable in this enclosure and repeated exposure to it within this sequence will ensure that is the case.

A Decision Box run occurred as below:



B versus C
flavour
choice

(5 min)

The animal was placed in the decision box which contained flavours B and C. The animal remained in this environment for 5 minutes before it was removed and returned to the home cage.

It is necessary that the animal is not only familiar with an empty decision box but also a decision box containing a flavour choice, as will occur during the later test phase. Therefore it is important that an animal is exposed just to the decision box containing a B vs. C flavour choice, as detailed above. It should be emphasised here that during Decision Box runs it is exclusively the decision box that is experienced, i.e., no context is experienced before or after. This is because an animal must not associate any context with a subsequent food choice in the decision box. In the later test phase an animal will be given an unexpected prior-choice after exposure to a context, however it is imperative that an animal does not become trained to this particular sequence. When an animal is offered a food choice it must be unexpected. If training runs were to combine contexts and flavour choices the animal may come to expect making a flavour choice.

Each animal experienced the training runs in a consistent order, such that after every 4 runs each rat had experienced 1A, 1B, 1C and 1 Decision Box run. This ensured that each animal was regularly exposed to every alternative, and that the animals became accustomed to making an occasional flavour choice in the decision box, though, crucially, not consistently, and not after having experienced any of the contexts.

The rats experienced three runs a day (morning, midday and afternoon) up to a total of 40 training runs. This meant 10 A runs, 10 B runs, 10 C runs and 10 Decision Box (D) runs. Five of the decision box runs had B against the outside and C against the inside wall of the area, and five vice versa to counterbalance any possible preferences of position within the context.

4.2b Procedure of training

Training followed a schedule, a sample of which is given below:

Table 4.1: Example of training schedule for all rats showing the contexts to which they were exposed, for the first 4 raining runs

Rat	**DAY 1**			**Day 2**	
	Run 1 (morning)	**Run 2 (midday)**	**Run 3 (afternoon)**	**Run 4 (morning)**	**Run 5 (midday)**
1	A	B	C	D	
2	B	C	D	A	
3	C	D	A	B	
4	D	A	B	C	
5	A	B	C	D	
6	B	C	D	A	
7	C	D	A	B	
8	D	A	B	C	…etc
9	A	B	C	D	

10	B	C	D	A	

Each rat's individual schedule meant that several rats could run at the same time (for instance, training run 1 above could include rats 1, 2, 3 and 4 in the apparatus simultaneously as these animals' runs all involve different contexts). However only cage mates were ever tested simultaneously and no context was visible or accessible from any other. Whenever animals were tested simultaneously like this, their start times were staggered by five minutes to prevent more than one rat being required in the decision box at the same time. None of the animals appeared distracted by any activity happening simultaneously in other contexts.

Fig. 4.2: Part of training schedule for cage 1, run 1, demonstrating the movements of rats 1 – 4 across 5 minute blocks. The shaded boxes show when an animal was in the decision box, to demonstrate that due to the staggered start times, only one animal was ever required in this area at any one time.

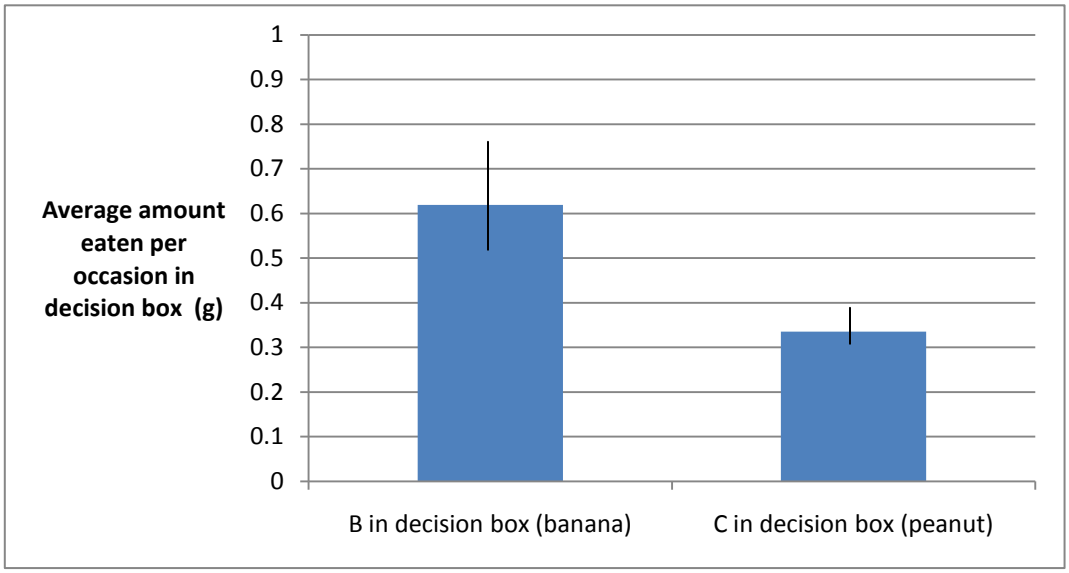
Rat	0-5min	5-10min	10-15min	15-20min
1	Empty context A	Empty Decision Box	Context A + flavour A	
2		Empty Context B	Empty Decision Box	Context B + flavour B
3			Empty Context C	Empty Decision Box…
4	Decision box + B/C flavour choice			

The rats were moved between the contexts and decision box as in Fig. 4.2 above. Rats always followed food trays into any context. All activity was filmed and all amounts of every flavour eaten were recorded.

4.3 Results of training

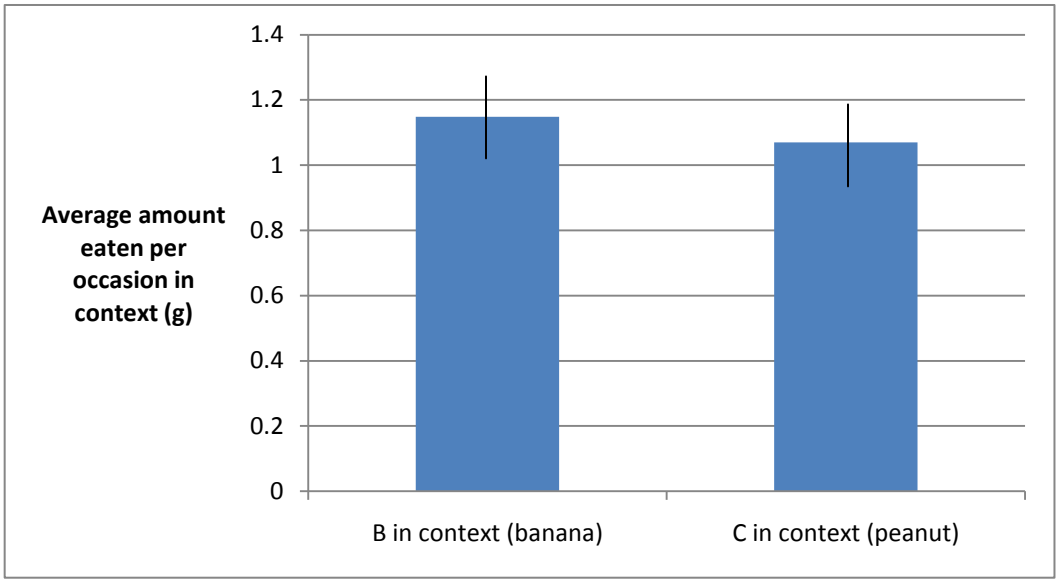
As this period was one of training there were limited results to report at this stage, apart from monitoring the amount of different favours eaten in context and decision box, to ensure strong preferences were not being developed

Fig 4.3: Mean amounts of flavours B and C eaten in per rat / per session in the decision box



As shown by fig 4.3, on average 0.62g of B and 0.34g of C was consumed per occasion in the decision box. A matched-pairs t-test showed that these means were not significantly different: **T_9 = 2.231, sig = 0.053** i.e., the two flavours were consumed relatively equivalently in the decision box here. It should be noted of course that this result is close to the significance threshold of 0.05, therefore the amounts of these flavours consumed will be monitored throughout the next stage of the study. The amounts of B and C consumed in context are shown below in fig 4.4.

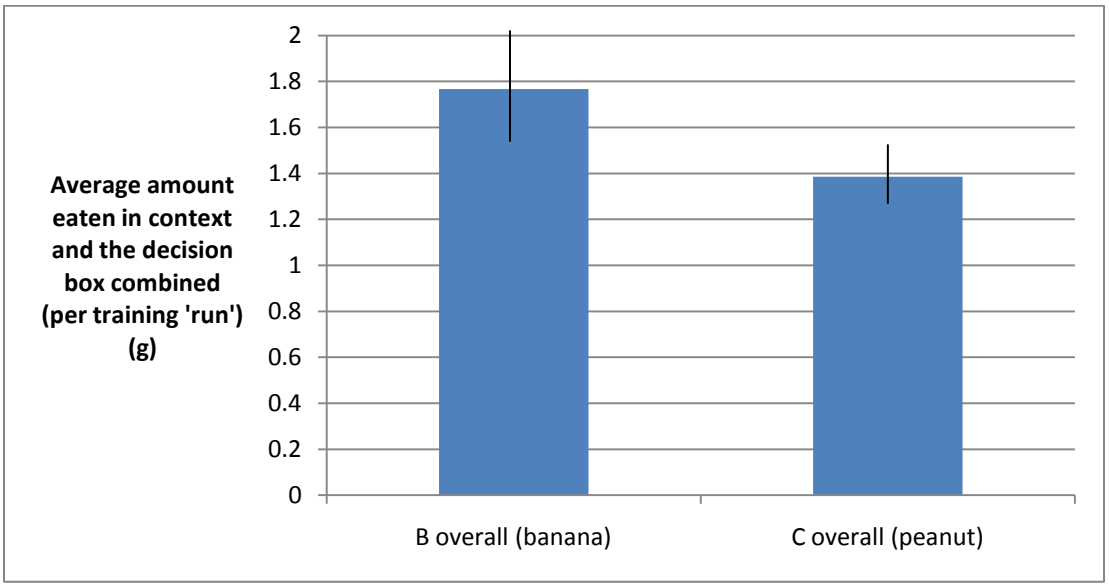
Fig 4.4: Mean amounts of flavours B and C eaten per occasion in context, per rat / per session



The mean amounts of B and C eaten in context (only one flavour present) were 1.15g and 1.07g respectively, shown above in fig 4.4. These means were not significantly different: **T_9 = 0.585, sig = 0.150** (matched-pairs t-test) therefore when taken in isolation the rats do not show a significant preference for either of the flavours.

In order to establish whether there was an overall flavour preference at this point, the average amounts of each flavour consumed in context and the decision box were combined, to give an average amount of each flavour consumed per rat, per training 'run'. As shown in fig 4.5 below, the average amount of B consumed was 1.77g and average amount of C 1.39g. A matched-pairs t-test demonstrated these means were not significantly different: **T_9 = 1.781, sig = 0.109.**

Fig 4.5: Mean amounts of flavours B and C eaten in context and decision box combined, per rat / per training run



4.4 Discussion of training

Observation of the animals made it apparent that they became more comfortable with the training procedure as time went on. After a short number of trials the animals showed far fewer occasions of stressful behaviours such as freezing, running in short frantic bursts and urination in the enclosure – all cited by Rodgers & Dalvi (1997) as indications of anxiety in the rat. Instead the animals showed interest in their surroundings with slow exploration of the walls and floor, standing on their hind legs and appearing unaffected by any unexpected noise.

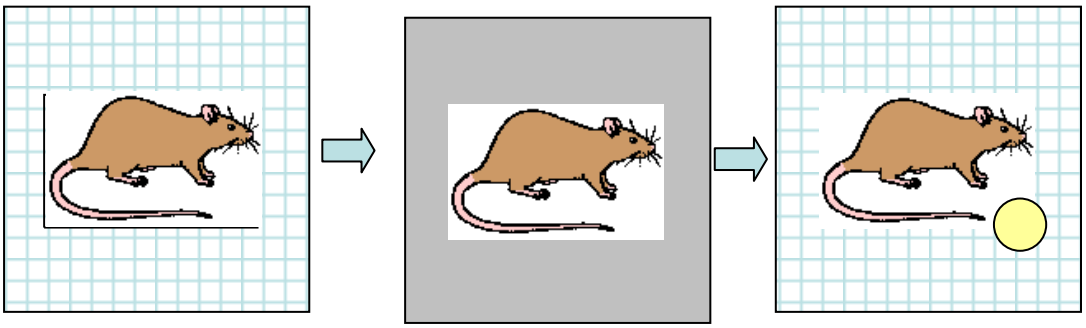
Looking at the mean amounts of B (banana) and C (peanut) consumed in the decision box, there was no significant difference in the consumption of the two flavours when given this choice, with the group consuming relatively similar amounts of each flavour. When considering the mean amount consumed of the same two flavours when in the associated contexts, there was also no significant difference here. To assess overall flavour preference, the *total* amount of each flavour consumed per rat, per training run (i.e. both in context and the decision box) was compared. A non-significant result here shows the animals on average do not prefer one flavour over the other. This is a positive result as it should ensure that any change in consumption is motivated by satiety-based anticipation, rather than a baseline flavour preference.

If these animals are capable of anticipatory SSS, in the testing trials to follow they will make a decision box B/C flavour choice based on the flavour they are expecting to experience. A rat demonstrating a future planning ability would for instance choose flavour B in the decision box if it had previously been exposed to an empty context C, in anticipation of the opportunity to soon become satiated by associated flavour C when returned to this context.

4.5 Method – testing

4.5a Design

The design remained almost identical to the training runs above. The animals were rotated through the same three runs a day, each rat still consistently rotated through A, B, C and D runs as before:



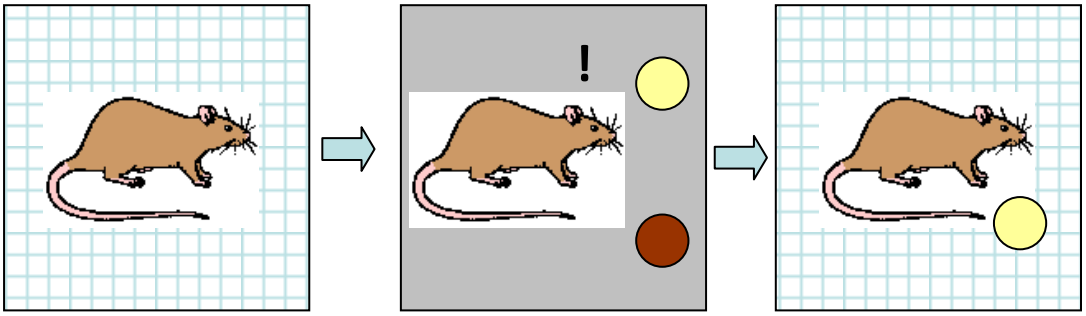
5 min in empty context 'X'

5 min in empty decision box

Returned to same context 'X' with appropriate flavour for 5 min

The main alteration to the procedure was the introduction of ***critical tests*** every nine runs, such that the first, ninth, eighteenth, twenty-seventh, thirty-sixth and forty-second run for each rat was a critical run. All other runs were 'fillers', and occurred just as the training trials.

During a critical test an animal would experience an A, B or C run just as in training, **though with the addition of an unexpected prior-choice present in the decision box**, as below:



5 min in empty context

Unexpected choice in decision box (5 min)

Returned to context with appropriate flavour for 5 min

The previous chapter demonstrated that, in the mornings, animals ate significantly greater amounts of the food to which they were first exposed, compared to that experienced subsequently. In the case of critical testing trials (as seen in the diagram above), the first 'food' experienced by an animal would be the flavour choice in the decision box. While the *difference* between the amount of food consumed in the decision box and that consumed on return to context is not a critical one here, the result in the previous chapter suggested an initial eagerness towards the first food experienced that was considered useful to exploit during the training trials. To take full advantage of this, all critical trials would be carried out in the morning.

It would be during these critical tests that the animals' future planning abilities would be assessed. The unexpected B/C flavour choice was only offered every 9 trials to ensure the rats would not come to predict its occurrence. It was essential that the animals did not expect having to make a choice until

they were in the neutral decision box. An *expected* test would be problematic for two reasons. The first is that it may result in the animal choosing which flavour to eat *whilst still in the empty context*. While this could be considered an example of forward planning it itself, it actually means that the flavour choice would be considered and/or made *whilst in an environment associated with a certain flavour*. This means the choice would be based on a state presently being experienced by the animal rather than an anticipated future state, and therefore could not be considered an example of true future planning. The second problem with a regular and therefore expected test was the potential for the rats to *learn* to choose the incongruous flavour based on becoming familiar with the increased or decreased pleasantness of the subsequent context-flavour. It was important that each critical test could be considered in isolation of any context so that any incongruous eating could be attributed to genuine flexible planning and not simple reinforcement. While in the empty context the rat should anticipate the opportunity to later become satiated by the context's associated flavour, but should not be expecting the decision box choice. It is only when the animal is in a truly neutral context that it can then make a flavour decision based entirely on the anticipation of a future state of satiation.

4.5b Procedure

On a filler run, i.e., when there was going to be no unexpected flavour choice in the decision box, the animals were rotated through the contexts in an A-run, B-run, C-run, D-run pattern exactly as in the training runs. On a critical run, none of the animals experienced a D-run, as all would experience an unexpected flavour choice in the decision box on these occasions.

Fig. 4.6: Part of testing schedule for cage 1 **on its first critical run**, demonstrating the movements of rats 1 – 4 across 5 minute blocks. The grey boxes show when an animal was in the decision box

Rat	0-5min	5-10min	10-15min	15-20min	20-25min
1	Empty context A	Decision Box + food choice	Context A + flavour A		
2		Empty Context B	Decision Box + food choice	Context B + flavour B	
3			Empty Context C	Decision Box + food choice	Context C + flavour C
4				Empty Context A	Decision Box + food choice

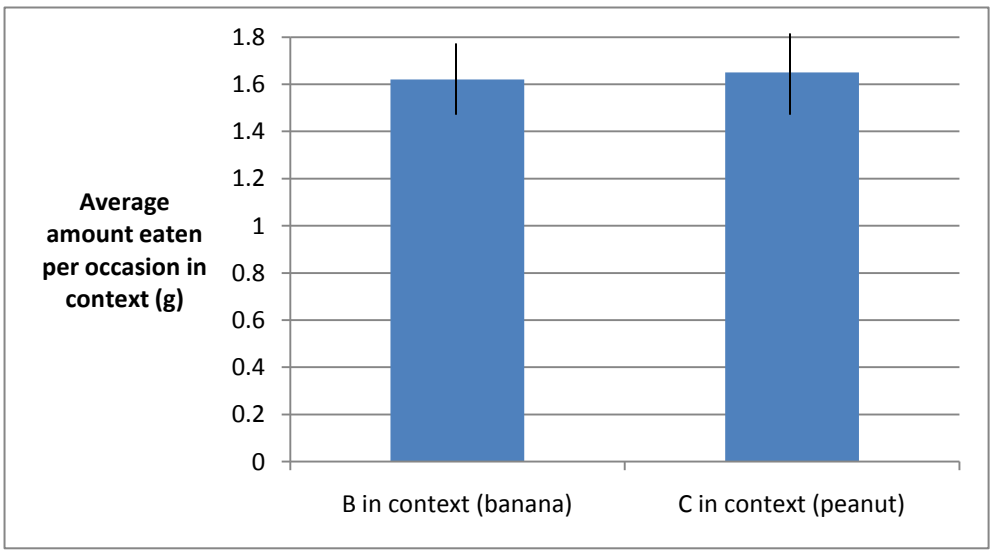
As demonstrated by figure 4.6 above, on the first training trial rat 1 was placed in empty context A for five minutes, and then transferred to the empty decision box as rat 2 was placed in empty context B. After five minutes rat 1 was returned to context A, now containing pellets of flavour A, while rat 2 was moved to the empty decision box and rat 3 placed in empty context C, etc. Rat 4 was placed straight into the decision box whenever it was unoccupied for five minutes, with a B versus C flavour choice. The following training session would run identically, although starting with rat 1 in empty context B, etc. In this way each cage of rats was rotated throughout a consistent sequence of empty context > empty decision box > context + food. Rats always followed food trays into any context. All activity was filmed and all amounts of every flavour eaten were recorded.

There were six critical tests for each rat, meaning each animal experienced 2 critical A runs, 2 critical B runs and 2 critical C runs to ensure equal experience of all alternatives. The position of the B and C food trays in the decision box were counterbalanced to ensure equal experience of each flavour on the left and right.

4.6 Results of testing

As mentioned previously, the average amounts of B and C consumed in context and in the decision box were calculated in order to check for a flavour preference. Fig 4.7 below shows the average amounts of each flavour consumed in context.

Fig 4.7: Average amounts of flavours B and C consumed in context per rat / per session



As can be seen from fig 4.7 above, the average amount of B and C consumed in context across the testing trials is very similar: B (banana) = 1.62g, C (peanut) = 1.65g. These means are not significantly different (matched-pairs t-test): **t_9 = -0.555, sig = 0.59.** The average amounts consumed in the decision box were also examined, shown below in fig 4.8.

Fig 4.8: Graph demonstrating the average amounts of flavours B and C consumed in the decision box, per rat / per session

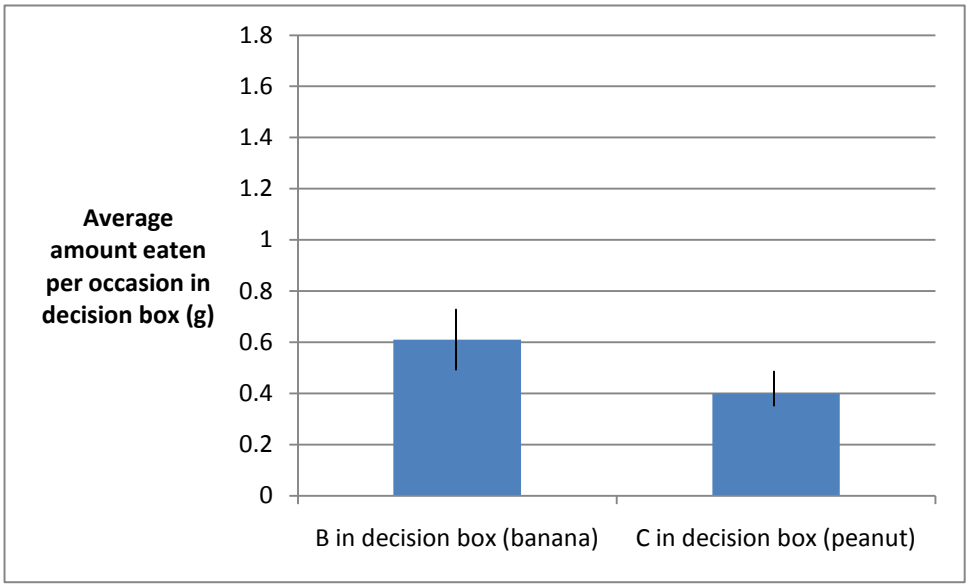
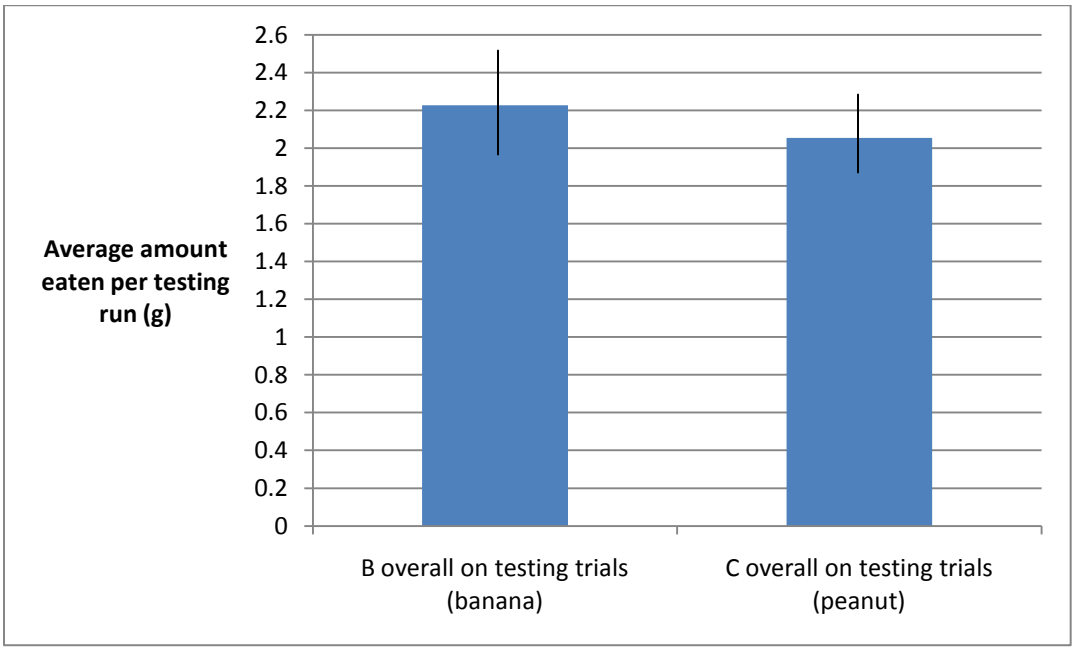


Fig 4.8 (using the same axis as previous fig 4.7, for comparison) shows that per occasion in the decision box, each rat consumed an average amount of 0.61g of banana and 0.40g of peanut flavour. These means were not significantly different (matched-pairs t-test): **t_9 = 1.137, sig = 0.29.**

In order to establish whether the animals displayed a flavour preference during the testing trials, the average amount of banana and peanut consumed per rat, per testing 'run' (i.e. the combined amount consumed in context and in the decision box) was calculated. The results are shown in fig 4.9 below. On

average, each animal consumed 2.23g banana and 2.05g peanut. A matched pairs t-test demonstrated these means were not significantly different: $\mathbf{t_9 = 0.841, sig = 0.422.}$ This shows an absence of a flavour preference during the testing trials.

Fig 4.9: Graph showing the average combined amounts of flavours B and C consumed in the decision box and in context, per rat / per testing run



Critical test data

The critical test data were processed in much the same way as the 'normal' SSS data -that is, using incongruity scores. If the animals demonstrated anticipatory SSS, they would choose to eat the decision

box flavour to which they were not expecting to be subsequently exposed. For instance a rat on a critical B run would placed in empty context B for 5 minutes and would enter the neutral decision box expecting firstly an empty decision box and secondly that flavour B will soon be readily available, as had been consistently the case for many weeks of training. The decision box would in fact unexpectedly contain flavours B and C. A rat successfully planning for the future would choose to eat incongruously, i.e., here would favour flavour C over flavour B, anticipating the opportunity to eat flavour B to satiation later.

The more positive the incongruity scores, the greater the tendency of the animals to eat the flavour they are *not* expecting, suggesting a future planning ability. The results for each rat and the group average are shown in table 4.9 below.

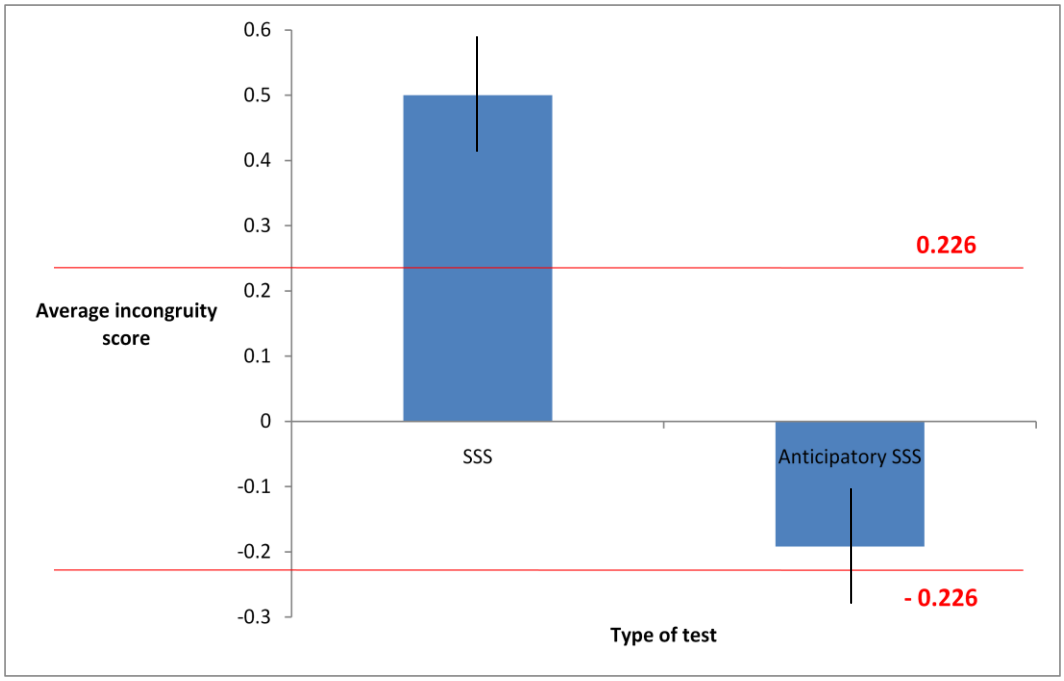
Table 4.9: Incongruity scores for each rat, averaged across six critical test runs

Rat	**Incongruity score**
1	-0.026
2	-0.333
3	-0.236
4	-0.208

5	-1.000
6	-0.046
7	0.250
8	0.000
9	-0.166
10	-0.154
Average	**-0.192**

A matched-pairs t-test was conducted on the above data as previously, comparing the average score with the zero score that would have occurred if all animals had eaten identical amounts of the congruous and incongruous flavour. The t-test showed that the average incongruity score above (-0.192) is not significantly different from the average zero in either a positive or negative direction: **t_9 = 1.860, sig = 0.096**, i.e., the average score is not significantly different from that which would be obtained if the animals on average showed no preference for either the congruous or incongruous flavour in the decision box. This group mean therefore does not support the hypothesis that the animals consumed the incongruous significantly more than the congruous flavour, and does not suggest that these animals are capable of future planning. Looking at the individual scores in the table above it appears that most are negative and fairly close to zero, meaning that at an individual level there does not appear to be a tendency to eat either congruously or incongruously.

Fig 4.10: Graph demonstrating the average group incongruity scores for SSS and anticipatory SSS



The red horizontal lines on fig 4.10 above show the threshold scores necessary for statistical significance to be achieved, i.e., an incongruity score of plus or minus 0.226 would mean that the score was significantly different from zero using a one-sample t-test. As seen in graph 4.10 above, when assessing SSS the incongruity score calculated fell above this threshold (in fact reaching 0.5), meaning that the animals consumed significantly greater amounts of the incongruous as compared to the congruous flavour, thus demonstrating SSS. The incongruity score obtained from the experiment to establish anticipatory SSS was -0.192. As seen from graph 4.10 this score fell between the upper and lower threshold levels meaning neither the congruous nor the incongruous flavour was consumed significantly more.

As shown in fig 4.9, the average amounts of B and C consumed per rat, per testing run (i.e. the amount in context and in the decision box combined) during these testing trials were not significantly different. Therefore it can be taken that (as opposed to the first SSS result in chapter three) a lack of significance in the anticipatory SSS test here is not due to a flavour preference. It was therefore decided to look at the amount eaten *in context* following congruous and incongruous eating in the decision box (for the critical trials in which there was an unexpected flavour choice in the decision box). This information may be useful as it should be this return to context (and its associated flavour) that the animals are anticipating when a choice is made in the decision box, therefore looking at the amount eaten here may provide an insight into the reasons for the animals' lack of anticipatory SSS.

Table 4.11 below shows the average amount of food consumed in context following a critical test in the decision box. The middle column shows the amount of food consumed following an incongruous food choice in the decision box and the right-hand column shows that following a congruous flavour choice (i.e., only food consumed in context B or C is included here). It would be intuitive to expect greater amounts to be eaten in context following an incongruous flavour choice, as the purpose of this choice would be to maintain the pleasantness of the upcoming context-flavour, in order that this flavour would be more pleasant to eat. However this was not the case, as shown below.

Table 4.11: Mean amounts consumed by each animal in context, following either an incongruous or a congruous choice in the decision box during critical testing days

Rat	Mean amount consumed in context after **incongruous** eating in decision box	Mean amount consumed in context after **congruous** eating in decision box
1	0.15	0.2
2	0	0.4
3	0	0.6
4	0	0.53
5	0	0.05
6	0.13	0.2
7	0	0
8	0.95	1.3
9	0	0.133
10	0	0.05
Average	**0.12**	**0.35**

A matched-pairs t-test on the data in table 4.11 showed that on average the animals consumed a significantly greater amount of food on return to context if they had previously eaten congruously in the decision box (0.35g subsequently eaten in context on average) than if they had eaten incongruously (0.12g subsequently eaten in context on average): **t_9 = -3.141, sig < 0.012.** While it is merely anecdotal,

it is interesting to consider the numbers of congruous and incongruous eating occasions across the testing trials: there were 40 critical runs on which it was possible to make a strictly congruous or incongruous choice in the decision box, i.e., on B and C runs (on the 20 A runs, both of the flavour choices in the decision box are 'incongruous' compared to flavour A). Of these 40 B and C runs, only 14 resulted in incongruous choices. Coupled with the 9 occasions of eating nothing when returned to context, this means there were just 5 occasions out of a possible 40 in which an animal made an incongruous flavour choice in the decision box and then went on to eat the supposed 'anticipated' flavour when returned to context. This is the behaviour that would be required to demonstrate nonhuman anticipation, and the rarity of such behaviour does not provide support for a planning ability in this group of animals as assessed by this method.

4.7 Discussion of testing

Relative amounts of congruous / incongruous flavour consumed in decision box

The average incongruity score calculated earlier in this chapter was not significantly different from zero, meaning that on critical trials relatively equivalent proportions of the congruous and the incongruous flavour were consumed. This means anticipatory SSS did not occur and means the current group of animals did not demonstrate an ability to plan for the future, which should have resulted in greater amounts of the incongruous flavour being consumed in the decision box in order to maintain the pleasantness of the anticipated context flavour. This is based on the assumption that consuming a certain flavour makes it subsequently more pleasant to consume an alternative – indeed this (SSS) was

demonstrated by the current group of animals in the previous chapter, when a higher proportion of the incongruous than the congruous flavour was consumed in the decision box following exposure to a certain context, and that context's associated flavour. It may merely be the future planning aspect of the current procedure which results in difficulty for this group of animals, or there may be other reasons for the lack of a significant result. Other findings will now be discussed in an attempt to understand the animals' behaviour.

Returning to context: Amount eaten following congruous/incongruous eating in the decision box

If an animal was capable of or inclined towards future planning in the form of anticipatory SSS, it could be assumed that the animal would consume more food on its return to context if it had previously eaten *incongruously* in the decision box. Congruous decision box eating could be considered to have satiated the rat to the flavour to which it is then exposed in context, resulting in a smaller amount being eaten due to the now reduced pleasantness of this flavour, in line with SSS (Rolls, 2005). However the opposite occurred in the current study. On average, an animal that made a congruous choice in the decision box then ate a larger amount when subsequently returned to context than when an incongruous choice had been made in the decision box. This result is unexpected in terms of the SSS literature (Rolls, 2005; Johnson & Vickers, 1992, Rolls, Rowe & Rolls, 1982, 1982), which shows that the perceived pleasantness of a certain flavour is reduced with continued consumption. The current results suggest that even if the animals are capable of anticipating the upcoming flavour (and there is no evidence of this ability), there appears to be *insufficient incentive* for them to demonstrate this planning ability by eating incongruously in the decision box, since when incongruous eating occurs it appears the animals are *less* inclined to eat when returned to context. The fact that a congruous choice in the

decision box does not deter the animals from continuing to eat this flavor on return to context suggests the animals have not been satiated by the amounts of food and timeframes allowed here.

This may seem at odds with the positive SSS result in chapter 3, which did demonstrate the occurrence of this characteristic reduction in pleasantness following consumption of a certain flavour. However this previous positive result need not have required 'true' satiation; a novelty-based flavour preference would have obtained the same result. *Anticipatory* SSS would have first required genuine satiety to be reached if the animals were to behave in response to an anticipation of this state. The rats varied in the amounts of food consumed, some eating the entire 2g presented and some less. In either case it is possible that genuine satiety may not have been achieved. The smaller amounts eaten may have been the result of the rats' ad libertum access to food throughout the period of the study. The literature on which this study's SSS theory was based often involved animals that were on a reduced diet, which increased the incentive to eat to satiation when they were allowed access to certain test flavours. The current animals may have never been entirely satiated by any of the flavours due to a lack of this kind of hunger drive. If this is the case they may still be capable of anticipating a future flavour, just not motivated sufficiently by an anticipated *satiety* to show a preference for the incongruous flavour in the decision box.

Returning to context: eating nothing

As aforementioned, in the present study, a rat successfully planning for the future would have eaten the alternative (incongruous) flavour in the decision box *in order that* it would not become satiated by the upcoming context flavour, such that when subsequently exposed to this flavour, its pleasantness would not have diminished and the rat would gain more enjoyment from consuming it. It seems counterproductive, then, that on over half of the occasions on which the incongruous flavour was selected in the decision box, the animal ate nothing at all when returned to the "anticipated" context.

One possible explanation may be that the animals were 'satiated' by food in general in the decision box, such that on return to context no further food is consumed. However evidence from critical trials in which animals ate the congruous flavour shows that this cannot be the case: following *congruous* eating in the decision box the animals are likely to eat more when returned to context than following incongruous eating, despite being returned to a flavour identical to that just consumed. It was therefore unlikely that the animals were actively anticipating the correct flavour even when they made an incongruous choice in the decision box, due to many of them failing to capitalise on their 'correct' choice by eating the supposedly anticipated flavour on return to context. This negative result is somewhat confirmed by the very few occasions on which an incongruous choice was followed by continued eating in context. A true planning capability would have resulted in more of such occasions.

Consistent incongruity did not occur, meaning a planning ability cannot be assumed in these animals. However it would be interesting to know if a rat could be capable of *learning* to consistently choose the

incongruous choice if exposed to critical trials much more frequently. An animal repeatedly exposed only to critical runs may start to assimilate information across continuing trials and learn that eating incongruously in the decision box would mean the subsequent context-flavour *is* more pleasant to eat (it should be, based on these animals' previous demonstration of SSS). An ability to learn this kind of future planning would still be an asset in a laboratory animal; consistently positive results may mean that exploratory surgery could investigate the processes involved in learning this complex cognitive activity. This may be similar to a young child's ability to learn to plan for his own personal future, or the possible re-learning experienced by someone affected by amnesia.

The final part of this investigation therefore centres on a 'learning test' to establish whether the current ten rats can learn to eat incongruously. Alternatively the case may be that the animals learn to eat congruously to an even greater extent, if this congruous behaviour produces a pleasant result for them. If learning is successful it would be expected that the group's average incongruity score would either drift in a substantially positive or negative direction.

4.8 Method - learning

The subjects and apparatus remained as above.

4.8a Design of learning test

The animals' ability to learn to anticipate flavours was assessed by altering the previous procedure such that now every run an animal experienced would be a critical run - i.e., a rat would be presented with a flavour choice every time it was placed in the decision box. In this way the flavour choice is no longer unexpected, and therefore the animals will begin to associate the now consistent choice in the decision box with their subsequent experience of being returned to context. Following repetitions an animal may alter its eating behaviour to take this new pattern into account. An animal may learn to choose the alternative flavour in the decision box based on the consistently repeated experience of making this choice and then being returned to context. After several trials an animal may associate the incongruous flavour in the decision box with being subsequently exposed to a flavour different to that which has just been consumed, which makes this second flavour more pleasant to eat.

The rats experienced one learning run every morning for 15 mornings, making 15 runs in total such that five A, five B and five C learning runs were included in each rat's schedule. There were no runs that featured only the decision box as there had been before, as the animals were now exposed to a decision

box choice every day, therefore it was considered that they did not require any further separate habituation to this environment.

4.8b Procedure of learning test

The procedure remained identical to the critical test runs described above, i.e., a rat would be placed in, for example, empty context B for five minutes, then transferred to the decision box in which there would be a B versus C flavour choice. Five minutes later the animal would be returned to the original context B which now contained pellets of flavour B (now considered a 'B learning run'). All behaviour was recorded with the same camera set up as in previous experiments, and all food eaten was recorded, also as before.

4.9 Results of learning test

If the repeated procedure allowed the animals to associate choosing the incongruous flavour in the decision box with a more pleasant eating experience when returned to context, it would be expected that the animals would eat more incongruously than congruously in the decision box. This would result in a positive incongruity score, potentially one above the necessary threshold to make the learning result significantly different from zero. If the incongruity score following the learning trials is more negative this would demonstrate the congruous flavour was eaten more than the incongruous and could

suggest the animals are instead associating the *congruous* flavour with a more pleasant return to context.

The average incongruity scores for each rat are shown and averaged below in table 4.12. As before, positive scores demonstrate incongruous and negative scores congruous eating in the decision box.

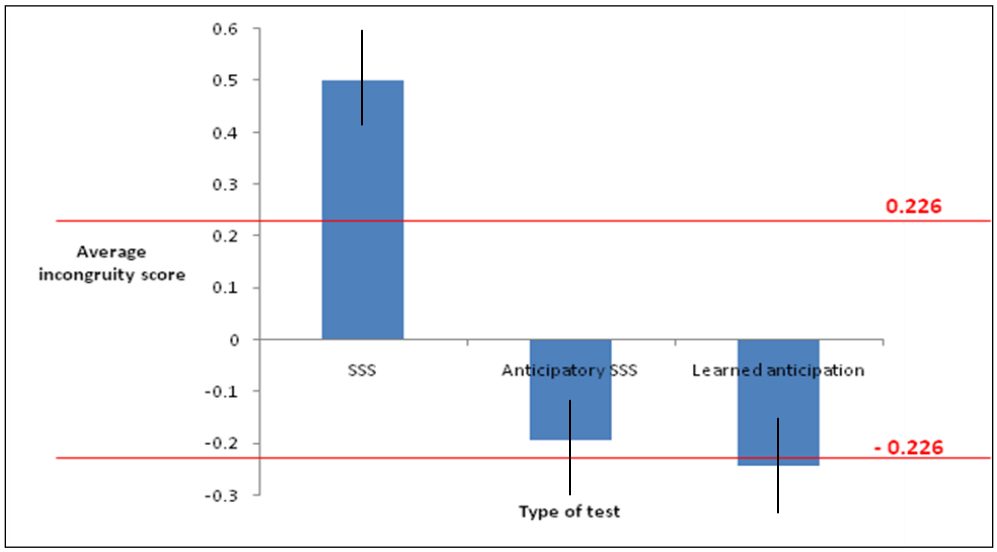
Table 4.12 Incongruity scores averaged for rats 1-10, averaged across 15 critical learning test runs

Rat	**Incongruity score**
1	-0.495
2	-0.003
3	-0.011
4	0.108
5	-0.589
6	0.004
7	-0.304
8	-0.104
9	-0.195
10	-0.841
Average	**-0.243**

The average incongruity score of the group is -0.243. This is more negative than the average score of the previous experiment (-0.192), and therefore does not demonstrate that these animals are able to learn that choosing the **incongruous** flavour presently ensures a more pleasant eating experience in the future.

However this score is significantly negatively different from the average incongruity score of zero that would be obtained had the animals shown no preference whatsoever for either the incongruous or the congruous flavour (matched-pairs t-test): $\mathbf{T_9 = 2.483, sig < 0.05}$. A comparison is shown on fig 4.13 below. The graph also displays red lines at the threshold levels at which an incongruity score would be significantly different from a score of zero (0.226 and -0.226). As the graph shows, the original SSS incongruity score (0.5) is significantly greater than zero, showing that on average the animals consumed significantly more of the incongruous relative to the congruous flavour. The average incongruity score from the tests of anticipatory SSS (-0.192) was not significantly different from zero. The incongruity score from the tests of learned anticipation was -0.243 and therefore above the threshold shown, meaning during these tests the animals consumed on average greater amounts of the congruous relative to the incongruous flavour.

Fig 4.13 the average group incongruity scores for SSS, anticipatory SSS and learned anticipation



Therefore in the most recent experiment (the 'learned anticipation' bar on the right of fig 4.13 above) the animals average incongruity score indicated that as compared to chance, the group as a whole consumes a greater proportion of the congruous as compared to the incongruous flavour. It appears then that these animals did respond to the learning trials, however they appeared to learn to associate consumption of the *congruous* flavour in the decision box with a more pleasant subsequent return to context. Possible reasons for this will be explored in the discussion section.

4.10 Discussion of learning

In the above learning procedure, animals capable of learning to plan for a future flavour would be expected to consume greater proportions of the incongruous over the congruous flavour. This would mean that on return to the anticipated context the animals would have access to a flavour different from that which they had just consumed. It was considered that this would be a more pleasant experience than being returned to a context containing a flavour that had been consumed very recently, based on the SSS data in chapter 3 of this study, and the large amount of SSS literature that describes this reduction in pleasantness following consumption (Rolls, Rowe & Rolls, 1981, 1982; Rolls, 2005; Johnson & Vicker, 1992; Bell, Rowe & Rolls, 2003). Moreover, throughout the learning trials the unexpected flavour choice was presented frequently, meaning the animals were able to learn **over several trials** the result of choosing the incongruous flavour in the decision box (subsequently exposed to an alternative flavour) and the result of choosing the congruous flavour (subsequently exposed to the same flavour). However over the course of the learning trials the animals consumed a significantly greater average proportion of the congruous than the incongruous flavour. This is counterintuitive as it means that an animal is then returned to a context containing the flavour which it has already eaten. According to the SSS literature, continued consumption of a particular foodstuff to the point of satiation results in its perceived pleasantness reducing relative to other flavours (Raby, Alexis, Dickinson & Clayton, 2007) textures and scents (Rolls, 2005), and even colours (Rolls, Rowe & Rolls, 1982). This could suggest that the amounts of pellets eaten in the current study were insufficient to satiate the animals during the testing and learning stages, meaning there was no real reduction in perceived pleasantness and therefore there was little incentive for the animals to seek out an alternative flavour.

Alternatively it is possible that the animals' eating behaviour during the learning trials was due to a genuine preference for congruous flavour consumption. Indeed, when testing for anticipatory SSS it was found that the animals ate greater quantities on return to context if they had made a congruous choice in the decision box. This suggests that some aspect of consuming the congruous flavour in the decision box (i.e., the flavour associated with the empty context which the animal experienced prior to the decision box) actually facilitates further consumption of this flavour when returned to the associated context – perhaps rather than coming close to satiating the animal, the flavour consumed whilst in the decision box actually 'primes' the animal to then continue eating the same flavour subsequently - perhaps related to the association found by Petrovich, Ross, Gallagher & Holland (in press), whereby an animal consumed greater quantities of a particular food while in a location associated with that particular food. If this is the case then it would make sense that this association begins when the animal is initially placed in the empty context at the start of its 'run': The location 'primes' the animal to eat the associated (congruous) flavour in context, which then does not deter the animal from continuing to consume this flavour when returned to context (particularly at this later stage of the study, by which time the animals were older and potentially capable of eating larger amounts before they became satiated).

4.11 Overall Discussion

Chapter 2 of this study was designed to establish the quantity of food and the timeframe required for the current group of rats to become satiated. This was important as the study was intended to identify an ability to anticipate a forthcoming satiation by a specific flavour, and an animal that has never experienced satiation could not be expected to anticipate this state. Despite this effort to investigate

the necessary criteria for satiety here, there are reasons why the resulting quantities and timeframes may not have been sufficient. Firstly the tests for satiety were carried out at the beginning of the study when the animals were younger and smaller. As the animals grew it is possible that the amounts of food initially sufficient to satiate the animals became less so as the rats gained in weight. The rats' ages is also something to be considered – Rolls & McDermott (1991) showed that adolescent (albeit human) subjects were much more susceptible to sensory specific satiety than older participants, i.e., consumption of a certain food by younger subjects produced a drop in pleasantness far more marked than in elderly participants, who were less likely to report feeling satiated by a certain foodstuff in terms of finding it less pleasant to eat; it is possible that as the rats aged throughout the study they experienced reduced levels of satiety. Another consideration is the lack of food deprivation amongst the current group of animals. Most investigations involving SSS require the subjects (human or not) to be at least partly food deprived prior to test. Some methodologies simply involve instructing human subjects to eat nothing between their breakfast and their lunchtime testing and then allowing the participants to consume as much as they want over a 15 minute period (Johnson & Vickers, 1992). Others instruct humans subjects that have eaten nothing since breakfast to consume a designated volume of a milk-based meal, in order to investigate the effects of volume and energy density (Bell, Roe & Rolls, 2003). Studies involving nonhuman subjects have induced satiety by allowing subjects 3 hours with an excess of a certain foodstuff (scrub jays - Correia, Dickinson & Clayton, 2007), or 20 hours of food deprivation followed by an excess of a certain foodstuff for 20-30 minutes (rats - Petrovich, Ross, Gallagher & Holland, 2007) or simply *ad libertum* access to normal 'home cage food' for 20 hours in order to satiate the animals to this food (rats - same authors). It is possible that the current animals did not experience satiety during the latter stages of the study, which may have rendered the group unable to anticipate this state with sufficient salience for it to guide their current behaviour.

Another possibility is that the animals are simply unable to do this task - it is enlightening that the learning stage as well as the testing stage was unsuccessful here as this does suggest that these rats cannot solve the task in any form, either spontaneously as a result of infrequent, unexpected tests or as a result of learning following a repeat procedure. It is also possible that rats are incapable of engaging in future orientated behaviour at all. It is true that at the time of writing no study has successfully demonstrated this kind of future planning in rats: Naqshbandi and Roberts (2006) tested rats and monkeys in order to establish if the animals would switch their intrinsic preference for a large pile of food to a smaller pile in order to speed the return of a water bottle to their cage. Crucially at the time of choice the animals were not thirsty, therefore success in the task depended on genuine anticipation of future state of thirst *not* currently experienced. After several trials the monkeys successfully chose the smaller pile of food and were rewarded with the swift return of their water bottle. The rats never learned to make this switch however, regardless of the number of trials experienced and the thirst that ensued, demonstrating an inability for this species to learn to solve a future planning task. Another study that attempted to establish a future planning ability in rats was McKenzie, Bird & Roberts (2005) work with a radial maze. Rats were allowed to store small pieces of food in the arms of a radial maze, and then to retrieve them after a certain period of time. The experimenters designated certain arms 'degrade' locations, such that any food stored here was inedible by the time the animals came to retrieve it. The rats never learned to avoid *storing* food in the degrade arms, though the animals did stop *retrieving* food from the degrade locations. This suggested an ability to remember which arms provide inedible food, yet these animals were incapable of using this information during the storing event. This suggests that the rats considered each act in isolation, i.e., did not consider storage to be connected in any way to later retrieval. The animals in the current study suggest a similar behaviour, as all but one of the animals alternated frequently between congruous and incongruous eating throughout

the entire 15 runs. This suggests that each run was considered in isolation rather than as one of a string of related experiences to learn from.

4.11 Summary

In summary, the current group of animals did not display spontaneous future orientated behaviour by choosing the incongruous flavour in anticipation of upcoming satiation in response to infrequent and therefore unexpected 'testing' trials, a behaviour shown by Correia, Dickinson & Clayton's (2007) jays. Neither did the group *learn* to display such future-orientated behaviour in response to frequently repeated 'learning' trials, in common with Naqshbandi & Roberts' (2006) rats. The learning trials resulted in a significantly greater proportion of the congruous flavour being consumed. The group therefore cannot be said to demonstrate anticipatory sensory specific satiety. While it is possible that the animals may have anticipated the upcoming flavour, this is not indisputably demonstrated by the behaviour shown, as memory and priming could be equally possible determinants of the behaviour shown in the learning trials. These unsuccessful results may be due to a lack of satiation throughout the study, resulting in the animals being unable to genuinely anticipate this state. Further reasons for this result are explored in the following chapter.

Chapter 5

Discussion

This study is one of many recent attempts to investigate whether nonhumans, in this case rats, are capable of planning for the future, i.e., whether nonhumans can engage in behaviour that is entirely future orientated rather than in response to the animals' present needs. In this way it re-examines Bischoff-Kohler's experimental conclusions (cited in Suddendorf & Corballis, 1997) that animals are "stuck in time" and aware only of their present motivational state. Tapping into genuinely future orientated behaviour requires a careful methodology and design in order to ensure that it is not confounded with behaviour that may result from current motivations. To this end, the present study utilises sensory specific satiety (SSS) (Rolls, Rowe & Rolls, 1981), in order to investigate the possibility of *anticipatory* SSS. SSS is the phenomenon whereby an animal exposed to excessive amounts of, and hence satiated by, a certain foodstuff will subsequently find that foodstuff less pleasant compared to alternative foods and will therefore select an alternative if offered a choice. It is a mechanism that promotes variety in the diet. Anticipatory SSS would occur if an animal was able to *anticipate* satiation, i.e., an animal would choose to eat a certain foodstuff (e.g., food A) *now* in order to prepare for an excess of a different foodstuff (e.g., B) in the future.

This ability to anticipate future satiation to a foodstuff was tested in the present group of rats. An animal capable of anticipating satiation to flavour A should, if offered a choice in advance, preferentially consume greater proportions of flavour B in order to preserve the pleasantness of anticipated flavour A – in accordance with the robust and widely established phenomenon of

SSS. The use of anticipatory SSS in this study helps to prevent current and future motivated behaviour becoming confounded because the incongruous choice holds no *present* benefit for the animals, hence consistently choosing the incongruous flavour must be taken as an indication of something *other* than present drives, thereby demonstrating future planning. This discussion will first consider what has and has not been demonstrated throughout the study; it will then explore how the results of the current study relate to other future planning literature. Finally, reasons for lack of success are explored, with possible improvements for future work.

What has and has not been demonstrated by the study

The study centred on whether or not the animals could anticipate future satiation. Therefore it was first important to establish if the animals would first demonstrate 'normal' satiation. In the early chapters of the study it was therefore necessary to investigate the conditions that would result in satiation in the current group of animals, e.g., timeframes, quantity of food required and the optimum flavours to use. Chapter 2 demonstrated that rats are capable of being satiated by a flavour in less than 5 minutes, indeed it was following 3-4 minutes of exposure to a certain flavour that the animals began to eat substantially reduced amounts of this flavour, demonstrating the characteristic reduction in pleasantness seen across the SSS literature (Rolls, 2005; Rolls, Rowe & Rolls, 1981;1982; etc). While human and nonhuman studies may not be directly comparable in this case, it is interesting to note that Smeets & Westerterp-Plantenga (2006) found that 5 minutes oral exposure to a certain foodstuff was also sufficient for humans to show SSS behaviour, i.e., to prefer an alternative foodstuff when subsequently offered a choice.

It was decided that in the upcoming satiation trials an animal would therefore spend 5 minutes in the presence of a satiating flavour.

The experiments in chapter 2 additionally assessed the amount of a certain flavour that was necessary and sufficient to satiate the animals during the previously established 5-minute timeframe. In the earliest trials the animals were provided with an excess of 5g of flavoured pellets, of which no more than half was ever consumed. The amount of food eaten was measured each day and averaged across the animals. With repeated exposure across several days the animals became habituated to the enclosure and consumed greater average quantities with each passing day. A plateau was reached from day 10 onwards, with the average amount consumed at 1.48g within the 5 minute timeframe. This was the average quantity of food that was necessary and sufficient to satiate these animals. It was decided therefore that a slight excess (2g) of food would be provided in subsequent trials in order to satiate the animals to a certain flavour.

Flavour preference was also investigated. In the initial experimental chapter (chapter 2), three flavours of Omnitreat^{tm} pellets were chosen at random (chocolate, tropical and peanut butter) and the consumption of each was assessed. It became apparent that the animals did not like tropical flavour, as the amounts consumed were significantly lower than the other flavours. Tropical flavour was therefore replaced with banana, which the animals consumed in much larger quantities. As aforementioned the study required two of the three flavours to be offered as a consistent flavour choice. It was important that these two flavours were equivalently preferable to the animals, to ensure that any differential consumption was the result of experimental

manipulations and not simple flavour preference. Initially chocolate and banana were paired together and formed the standard flavour choice, however it soon became apparent that animals substantially preferred banana. The paired flavour choice was therefore altered to consist of banana and peanut flavour pellets, which were more equally palatable and reduced the possibility that intrinsic flavour preference would drive eating behaviour.

In chapter 3 it was established that the current group of animals demonstrate SSS (Rolls, Rowe & Rolls, 1981). This means that following 5 minutes exposure to a certain flavour, e.g., banana, when subsequently offered a flavour choice between the flavour just experienced, and an alternative (e.g., peanut), the animals on average consume significantly higher proportions of the alternative (incongruous) peanut flavour, due to the reduced pleasantness of the banana pellets relative to other flavours.

It was also established that in the mornings, the animals ate greater amounts of the food first experienced than the food experienced subsequently (i.e., that the animals were particularly eager to consume the first experimental food experienced in a day). . While this difference in consumption between first and second food experienced was not a critical one when testing anticipatory SSS, it was considered reasonable nevertheless to take advantage of the animals' initial eagerness to eat the first food presented, as in the case of critical trials this was the all-important flavour test in the decision box. Therefore all critical testing sessions occurred in the morning. Chapter 3 also involved creating an association between pellet flavours and environmental contexts, for example, the rats learned through repeated exposure that peanut was always experienced while in the black apparatus, whereas banana was always experienced when in the light coloured enclosure. Various tactile floor coverings (mesh, PVC, etc) made the contexts even more distinct.

Chapter 4 investigated future planning. The animals were trained to anticipate a return to a certain environmental context by moving them between the different contexts and the decision box in a simple, repetitive sequence, for example: empty context A, followed by the empty decision box, followed by a return to context A (and context A's associated flavour of pellet). At test, the well-established sequence was interrupted with an unexpected flavour choice in the decision box, and prior to the animal's return to context and the context's associated flavour. The unexpected flavour choice consisted of the flavour associated with the upcoming context, and an alternative flavour. This design assessed whether, when given an unexpected choice, the animals would choose to eat proportionally more of the incongruous flavour in the decision box, demonstrating a proclivity to preserve the pleasantness of the anticipated flavour on return to context.

However this was not demonstrated. The animals showed no such tendency to consume the incongruous over the congruous flavour, in fact there was no significant difference between the average proportion of the congruous and incongruous flavour consumed in the decision box. This does not demonstrate awareness of any upcoming satiation. The animals' behaviour therefore did not demonstrate any examples of spontaneous future planning. Following this result it was attempted to establish whether the animals could *learn* to solve the task if the flavour choice was presented daily, rather than infrequently as before. This alteration meant that the animals had the frequently repeated experience of a flavour choice in the decision box followed by a return to context and the context's associated food. This repetition allowed the animals to learn from the consequences of their choice in the decision box with respect to the subsequent pleasantness of consuming the anticipated flavour in context, i.e., it was thought that an animal that ate the incongruous flavour at decision would be subsequently 'rewarded' on

return to context with a flavour that was more pleasant to eat, and the animal then has the opportunity to attempt the same reward-inducing behaviour the following day. Conversely, an animal that chooses the *congruous* flavour in the decision box would return to context to find the context flavour reduced in pleasantness due to the large amounts of this same flavour just consumed in the decision box. As before however, the animal has the opportunity to correct this 'mistake' the following next day by choosing to consume the incongruous over the congruous flavour in the decision box. The current group of animals did not learn to consume a larger proportion of the incongruous flavour however, and instead over the course of the learning trials consumed a significantly larger proportion of the congruous flavour in the decision box.

Consideration of similar studies

The fact that the animals can neither perform the task spontaneously *nor* learn to solve it is enlightening as it demonstrates these animals do not appear capable of solving this task in any form – either in terms of genuine future planning based on a one-off occasion, something demonstrated by Correia et al's (2007) scrub jays, or *learning* to anticipate following repeated exposure. This is interesting in terms of the future planning literature, as the latter, learning, stage of the study was similar to a study by Naqshbandi & Roberts (2006) in which monkeys and rats were offered a choice between a larger and a smaller pile of food. Choosing the larger pile was considered to be ultimately the more presently rewarding, however choosing the smaller pile meant a water bottle was returned to the cage sooner than if the large pile was chosen. The food

made the animals thirsty, and after a number of repeated trials the monkeys were able to anticipate this upcoming thirst and switch their preference from the larger to the smaller pile of food. The rats however never learned to switch their preference and continued choosing the larger pile of food regardless of the thirst that occurred every time. Success in this experiment relies on the animals making a choice that could be regarded as negative for their current self, but advantageous for their future self. This means the animals had to compare the benefits of the current choice (large pile of food) with the benefits of a future situation (swift return of water bottle preventing uncomfortable thirst).

Success in the present study would not have required the animals to make a choice that was negative for their current self, as the two flavours offered were equally palatable to the animals, and so no such present-versus-future trade-off was necessary. This potentially made the current task easier to solve than that of Naqshbandi & Roberts (2006), and yet still the rats were unable to learn it.

Other studies have had similarly little success in demonstrating the *learning* of future planning over a number of trials in rats. McKenzie, Bird & Roberts (2005) repeatedly allowed rats to freely cache small pieces of food in any of 8 arms of a radial maze. Four of the arms were designated 'degrade' locations, such that any food stored in those arms by the rats was rendered inedible by the time the animals came to retrieve their caches. Any food stored in the 'safe' half of the maze was still good to eat on retrieval. This experiment was repeated many times, and yet the animals did not learn to preferentially store food in the 'safe' arms of the maze, rather, the storage locations remained random. Of note was the fact that when it came to retrieving their caches the rats only *searched* for food in the safe arms, demonstrating they understood the

difference between the safe and degrade locations at retrieval, but were not capable of using this knowledge when storing. It appears that they were not capable of associating storage with the future activity of retrieval, even after several repetitions.

Taken together, the results of these studies and those of the current investigation suggest that rats are simply incapable of solving future orientated tasks, or indeed learning to do so. However it should be noted that there are relatively few studies that have successfully tapped into an ability to behave in a way that is entirely future orientated. For example Mulcahy & Call (2006) investigated future planning by assessing the abilities of chimpanzees to choose a tool that would enable them to extract grapes from a machine on a future occasion, however the apes were offered a choice of tool while within sight of the apparatus on which the tools would be used, therefore there was no guarantee that the animals were basing their choice on future rather than current drives to extract grapes. This is similar to the studies of episodic memory that do not always make the necessary explicit distinction between action based on current drives or familiarity, and action based on genuine recall of a past experience, for example Schwartz, Hoffman & Evans (2005) tested King the gorilla's memory of exposure to certain foods or carers. However the authors acknowledged that the regular testing sessions may have allowed King to 'prepare' for his test by storing his experiences of foodstuffs or certain carers as something akin to semantic knowledge, ready to communicate at test as something that he simply 'knows' rather than genuinely 'remembers', in order to get a food reward. Similarly, Eacott & Norman (2004) acknowledged that testing rats' memory for certain objects by assessing their behaviour towards these objects in an open field, allows the animals to use current feelings of familiarity versus novelty, rather than genuine memory, to solve the task. The only

demonstration as yet of true nonhuman future-orientated behaviour (spontaneous rather than learned in this case) appears to be Correia, Dickinson & Clayton's (2007) investigations, which also utilise the concept of anticipatory SSS. What makes this study interesting is that the task required scrub jays to make a choice *against* their current motivations as in the Naqshbandi and Roberts study, rather than making a neutral choice, as in the present study. Correia et al's (2007) scrub jays were exposed to a certain foodstuff (A) for 3 hours, resulting in complete satiation to that foodstuff. The birds were then given a choice of this foodstuff (A) or an alternative (B) to cache, for retrieval later. The birds were taught that the future retrieval of this cache would follow 3 hours of exposure to foodstuff B.

Therefore initially caching foodstuff A would ensure that the pleasantness of the retrieved flavour remained high. However this required the birds to cache foodstuff A *while satiated by this flavour*, which is behaviour against the birds' current satiation-based drive to select foodstuff B. This group of jays were successful because the birds chose on average to cache more of foodstuff A despite being currently satiated by it. This demonstrates an awareness that the current caching event was linked to the future retrieval event, a connection that McKenzie et al's (2005) rats were incapable of making, even following repeat trials. This demonstration of apparently truly future orientated behaviour means the Correia et al (2007) study has come the closest to demonstrating nonhuman future planning. It has been commented previously however (Eacott & Norman 2005) that it would be particularly useful to observe similar abilities in a species that does not so instinctively cache food for future retrieval, hence these authors' investigations into the abilities of rats. It remains the case that no study has yet demonstrated the same future planning ability in rats, through truly future orientated behaviour. There is the possibility of course that this and other species simply do not have this capability.

However, it should be remembered that there are several studies that have successfully demonstrate the episodic-like memory abilities of animals – the ability to behave in response to the genuine recall of a complete memory (Clayton, Bussey & Dickinson, 2003; Zentall, 2005), including rats (Eichenbaum & Fortin, 2003; Eacott, Easton & Zinkivskay, 2005). This is interesting because much developmental evidence (Atance & O'Neil, 2005; Suddendorf and Busby, 2005) as well as patient studies (Klein, Loftus & Kihlstrom, 2002; Hassabis, Kumaran, Vann & Maguire, 2007) and neuroimaging studies (Botzung, Denkova & Manning, 2008) strongly suggest that episodic memory in humans is one half of an over-arching *mental time travel system* which also encompasses the ability of future thinking. This 'Mental Time Travel' system (Suddendorf & Busby, 1997) allows humans to consciously travel in either direction along their own personal timeline – i.e., to recall their past and imagine their future. If episodic memory and future thinking do in fact rely on the same cognitive processes, then the demonstration of nonhuman episodic-like memory in any species (the ability to behave in response to genuine recall of the past) should mean this species is also capable of future planning (behaviour in response to a genuinely anticipated future). Indeed, Tulving (1999, cited in Atance & O'Neill, 2001) makes the point that the role of episodic memory (recalling the past) is largely to enable a creature to use this special kind of memory in order to plan for an uncertain future. For example, Tulving suggests that the ability to vividly recall an unpleasant past episode must have little use beyond ensuring that such an event does not happen again, and therefore there would be little advantage for an animal to possess the retrospective ability if it was not to be used in tandem with a prospective planning ability.

The results of the current study certainly suggest that rats are not capable of solving the current task, however the above cited evidence means it would almost be counterintuitive to assume that this group of rats would be incapable of *any* task requiring future planning. Therefore it should be considered why the current study may have been unsuccessful, leaving aside for now the possibility that rats simply do not have this ability.

Lack of success in the current study

The current study was unsuccessful in demonstrating a future planning ability based on two results: Firstly, during the testing phase (in which the animals were presented with infrequent and unexpected flavour choices) the rats did not choose to consume proportionally more of the incongruous flavour, as would be expected of animals capable of anticipating satiation to a particular flavour in the near future. There was no significant difference in the proportions of incongruous or congruous flavours consumed at this stage. Secondly, in the learning phase (in which the animals were presented daily with a flavour choice) the rats did not learn to consume greater proportions of the incongruous flavour, even following these repeated trials. In fact, over the course of the learning trials, the animals consumed a significantly higher proportion of the *congruous* flavour when in the decision box. This demonstrates that the animals did appear to shift their eating behaviour from consuming equal proportions of the two flavours in the test trials, to the significant preference for the congruous flavour during the learning task. While this does appear to demonstrate learning of a sort, it is learning to consume more of the *incongruous* flavour that would have suggested an ability to plan for upcoming satiation. The current result shows that consuming, for example, peanut flavour in the decision box does not diminish the

pleasantness of consuming yet more peanut flavour pellets on return to context. Therefore the following questions should be considered: firstly, what possible reasons are there for the animals' inability to solve, or to learn to solve, the task, other than simply that rats are incapable of any kind of future planning. And secondly, what reasons are there for the result obtained here: a greater proportion of the congruous flavour consumed during the learning phase, as this behaviour could be interpreted as somewhat counterproductive.

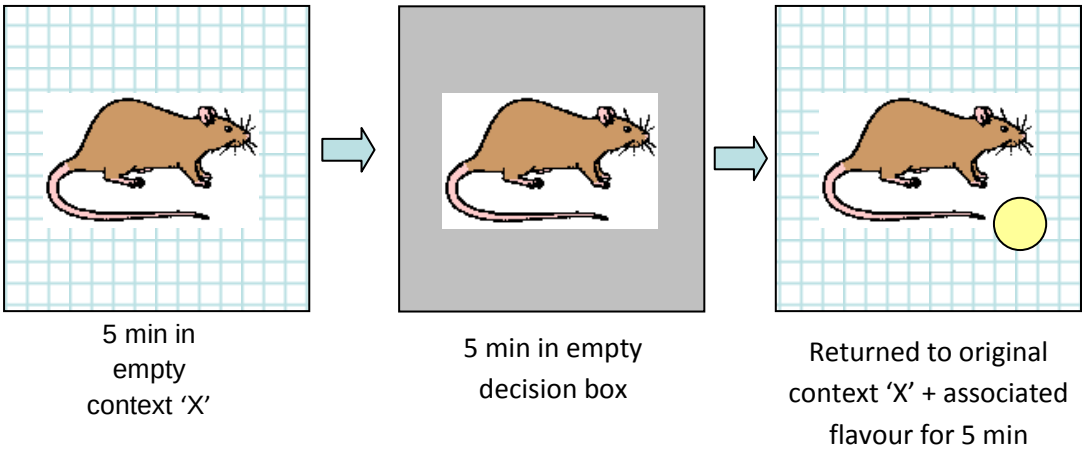
In terms of the lack of success in the task, the study has made a number of assumptions that may not in fact be appropriate, and may offer an explanation as to the lack of success in this task. One such assumption relates to the associations between the contexts and flavours. This study of anticipatory SSS relied on the animals associating certain contexts with certain flavours. This association was attempted by only having the animals experience certain flavours in certain environmental contexts, for example, the animals only ever experienced chocolate flavour pellets when in context A. This meant that an animal anticipating a return to context A (following the much repeated sequence of Context A > decision box > Context A) should *also* anticipate upcoming exposure to chocolate flavoured pellets. It was on the assumption that context A *was* associated with chocolate that a flavour choice was offered in the decision box prior to the animal's return to this context. However an animal that had made no such association would not anticipate chocolate flavour pellets and so would not be able to make a flavour choice in the decision box based on anticipatory SSS (even if the upcoming context was successfully expected). Therefore the inconclusive result obtained here may have been the result of the animals not making these context-flavour associations. However, the results from the later, learning phase of the study suggest that such an association *was* in place: during the learning phase, when the animals were offered a flavour choice in the decision box on a daily basis, the

rats consumed on average more of the congruous than the incongruous flavour in the decision box. While this does not demonstrate future planning abilities, it does suggest that the animals were choosing to eat the flavour associated with the context from which they had just come. This result is in line with the aforementioned findings by Petrovich, Ross, Gallagher & Holland (in press) that an association between a location and a particular kind of food causes an increase in the consumption of that food when in the location. This congruous flavour preference was also not seen in the earlier testing trials, when the food choice in the decision box was only rarely presented. It may be the case that frequent experience of a flavour choice (which included the flavour associated with the context just experienced) in some way cemented this association. Alternatively, it is possible that the animals began to anticipate the (now frequent) flavour choice before they were placed in the decision box, allowing them to make a choice 'in advance' i.e., while still in the context associated with the congruous flavour, and therefore more likely to choose that flavour, as with the Petrovich result cited above. Therefore the congruous flavour preference seen in the learning trial does not demonstrate that the animals can plan for the future, but it does suggest that the animals at least had associated the various environmental contexts with the intended flavours.

Another assumption that was not sufficiently challenged in the present study was the animals' ability to genuinely predict the upcoming context. Even if the contexts were successfully associated with flavours, an animal incapable of anticipating which context it would next experience would be incapable of expecting satiation to the context's associated flavour. The learning results considered in the above paragraph do not suggest that the animals, when in the decision box, are anticipating being returned to context. It was attempted to train the animals to successfully anticipate the upcoming context by repeatedly exposing them to a simple sequence

of locations: placement in a particular context, followed by a short spell in the decision box, followed by a return to the original context (shown in fig 5.1).

Fig 5.1 – sequence training the animals to anticipate the upcoming context, and upcoming satiety



It is intuitive to assume that following extensive repetition of this simple sequence an animal would come to anticipate where it would be placed next, i.e., when in the decision box an animal should be capable of anticipating its return to the context it was in previously. This assumption is based on anecdotal accounts, field observations and more controlled experiments within the laboratory that demonstrate nonhumans are sensitive to habits relating to time and place: Roberts (2002) cites the example of a dog that approaches its food dish at meal times, as well as field studies that observed oystercatcher birds appear on the mudflats at the point of lowest tide each day, both of which show anticipation born of frequently repeated behaviour (though it is acknowledged that these behaviours do not provide evidence for genuine forward *thinking*). Even more relevant, and under controlled lab conditions, Biebach, Gordijn & Krebs (1989) found that garden warbler birds learned which one of many feeding rooms contained food at different periods throughout the day, such that after learning the sequence they moved flawlessly between the rooms each hour in order to gather the food. It is interesting to note that during the

present study, after 5 minutes in a particular enclosure, a rat would often move to the specific corner from which the experimenter consistently collected it. All of the aforementioned suggest that a variety of nonhuman species are capable of learning time-place sequences. It should of course be noted that the examples above involve learning based on habitual action on the part of the animal, rather than the passive movements of the current study.

It was never conclusively demonstrated that an animal in the decision box was capable of anticipating the remainder of the sequence, and its return to context. An animal that was unaware of its impending return to context A, for example, would have no anticipatory-based reason to consume the incongruous flavour in the decision box. This may have contributed to the lack of any significant flavour preference at the testing stage. The preference for the congruous flavour at the learning stage does not throw light on this issue, as this preference need not be based on anticipation of the upcoming context, rather the same result could occur from memory of the context just experienced, or simple priming.

This uncertainly as to the animals' context anticipation is a drawback of the study. A possible test for this, following many experiences of the above sequence, may have been to move the animal from the decision box to a context (and associated flavour) that was 'out of sequence' and observe the animal to establish if this move out of sequence created more occurrences of novelty-seeking behaviours than a return to the 'expected' context, such as increased exploration of the enclosure and food dish (containing the flavour associated with the relatively novel context), including standing on hind legs against the enclosure walls, sniffing all areas of the enclosure and increased overall distance walked within the enclosure. These are behaviours that Eacott and Norman (2004) and Dix and Aggleton (1999) successfully recorded as an indication of an animal's awareness of changes to the environment, used by Eacott et al to demonstrate that

rats are capable of noticing very slight alterations to an otherwise unchanged environment. A lack of a formal test for this ability is a drawback of the present study, as it is possible that when in the decision box the animals were not aware of their imminent return to the appropriate context and thus were unaware of which flavour they were soon to experience.

The third important assumption of the study was that any anticipation would for genuine *satiation* of the upcoming flavour, and not simply anticipation of 'exposure' to the context and flavour combination. The study intended to assess anticipatory SSS – the anticipation of a future state of *satiation* by a certain flavour, sufficiently salient to drive the animals to choose the alternative flavour if offered a flavour choice in advance. The initial experiments of chapter 2 were designed to establish the quantity of food and the timeframe required for the animals to become genuinely satiated, and this information was subsequently used in chapter 4 to train the animals to expect genuine satiation to a certain flavour: as shown previously in figure 5.1, an animal was returned to the appropriate context in which had been placed 2g of a certain flavour of pellet, to which the rat was exposed for 5 minutes. According to the results in chapter 2 this was sufficient to result in satiation, however the results from chapter 4, discussed below, suggest these animals were not satiated under these conditions and thus could not be expected to anticipate this state of genuine satiation during the testing phase of the study. It would only be anticipation of *satiation* by a certain flavour, rather than merely anticipation of the flavour itself, that would drive an animal to select the incongruous flavour in preparation.

In chapter 4 it was demonstrated that the rats consumed greater amounts of food when returned to context following a congruous flavour choice in the decision box. It may have been assumed

than an animal eating a large amount of the congruous flavour in the decision box would become satiated by this flavour, rendering the same flavour less peasant to eat immediately afterwards on return to context, however this was not the case. This suggests that by the later stages of the study, the suggested 2g of food used to satiate the animals to a particular flavour was no longer sufficient.

Conversely, it was also the case that the animals ate significantly smaller amounts of food on return to context following an incongruous flavour choice in the decision box, which is somewhat counterintuitive: it would be assumed that an animal eating incongruously in the decision box would be doing so in order to ensure that subsequently eating the anticipated flavour in context would be a more pleasant experience. However this did not appear to be the case. This seems at odds with the positive SSS result in chapter 3, in which the animals ate significantly greater proportions of the incongruous flavour (that which they had *not* just experienced), which would suggest that the animals *had* been satiated by 2g of the initial flavour, however these findings are reconcilable. In a successful demonstration of 'normal' (as opposed to 'anticipatory') SSS, the animals eat incongruously *following* exposure to a certain flavour, i.e., they must simply react to their current experience. Even if not entirely satiated by the original flavour it is likely that a rat would still prefer to subsequently sample a relatively novel one if given the choice, in accordance with rats' intrinsic preference for novelty over familiarity - hence the positive SSS result. However, anticipatory SSS is more complicated as it involves imagining future motivations rather than reacting to present ones. The mere expectation of a certain *flavour* may not be sufficiently salient for an animal to choose the incongruous flavour in advance. For this incongruous choice to occur, the animal may have to expect genuine satiation by a certain flavour, rather than expect simply the presence of that flavour. In fact, it makes sense that

expecting an upcoming flavour (but not expecting satiation to it) may result in the congruous choice being preferred in the decision box, due to this flavour being more salient at the time of decision.

As discussed before however, these animals do not appear to become truly satiated by 2g of food, or across 5 minutes, particularly during the later parts of the study that investigate future planning. This lack of satiation is obvious because an animal that consumes all 2g of the congruous flavour in the decision box does not subsequently appear to find the pleasantness of this flavour diminished on return to context, as would be the case if true satiation had occurred. This lack of satiation may be due to the increased size of the rats since the initial experiments to assess their satiety thresholds. These assessments were carried out when the animals were between approximately 3 and 4 months old. By the time the rats were tested at chapter 4 they were approximately 7 to 8 months old, hence the original food quantities and timeframes may have been insufficient by the time the rats reached this age. The increased age of the animals should also be considered – Rolls & McDermott (1991) obtained the result that young rats demonstrated a much stronger result in favour of SSS than older rats. Therefore an age-related decline in this tendency to seek relative sensory novelty may have hindered these animals in becoming satiated in the later parts of the study. If the animals were in fact never satiated by the context flavour it would be impossible for them to anticipate it as a future state during the critical tests.

It may be the case that genuine satiation can only be reached if the animals are previously food deprived to at least a certain extent, to facilitate increased consumption of a certain foodstuff or flavour. Most studies investigating satiation (including those involving humans), e.g. Guinard & Brun (1998), Hetherington et al (1981), Rolls et al (1983), ensure that subjects are deprived of food

for at least a few hours before test, as a genuinely hungry animal is more likely to eat a satiating amount of an experimental flavour when given the opportunity. The current animals never experienced hunger however, due to their ad libertum access to food throughout the study. Any further investigation of anticipatory SSS may wish to take this into account. It is interesting to note that the study by Correia et al (2007) which does appear to demonstrate future planning in scrub jays by utilising anticipatory SSS, does not involve food deprivation. However these birds were exposed to the appropriate satiating foodstuff for three hours prior to either caching or retrieval, which deprived the birds of all *other* kinds of food for a substantial period of time prior to test. This ensured that if the birds were hungry they consumed only the intended foodstuff for 3 hours, which resulted in the birds being completely satiated. It is likely that the current rats were never entirely satiated by the experimental flavours.

The above discussion offers two possible reasons for the animals' lack of success, namely the possibility that the animals did not learn the sequence of experimental contexts, and the possibility that the animals had not experienced genuine satiation and therefore could not be expected to anticipate it. However the result from the final stage of chapter 4, the learning stage, is not entirely inconclusive. As aforementioned, during the testing stage (during which the food choices were infrequent and unexpected) there was no significant difference between the proportions of congruous and incongruous food consumed at choice in the decision box. At the learning stage however (when the flavour choices were presented daily) the animals consumed a significantly higher proportion of the congruous flavour. It may be useful therefore to consider the differences between the two final parts of the study – the 'test' and 'learn' stages, in order to investigate why this preference for the congruous flavour developed. The one main difference

between the testing and learning stages was the frequency with which a food choice was presented in the decision box. As aforementioned, a food choice was presented infrequently during the testing stage (most of the time during this testing stage the animals experienced an empty decision box), but daily during the learning stage. The frequent food choices of the learning phase meant two things: firstly, the animals were able to frequently experience the *consequences* of the flavour choice they made, in terms of the subsequent pleasantness of the context flavour when they are returned to context. This provided the opportunity to learn to anticipate the increased pleasantness of the context flavour following an incongruous flavour choice in the decision box. Secondly, and perhaps more importantly in this case, the frequent flavour choice of the learning stage allowed the animals to come to *expect* that this choice would be presented, rather than the choice being unexpected as before. If an animal anticipates (while still in context) an upcoming choice in the decision box, it is possible that the upcoming decision will be influenced by the context the animal was presently in. This means the rats may have been responding according to the sort of conditioning that was seen in the experiments by the aforementioned Petrovich and colleagues (in press) - whereby an animal in an environment associated with a particular flavour is more likely to consume greater amounts of that flavour than when in an environment not associated with that flavour. If the current group of animals were in some way aware of the upcoming flavour choice while still in context, this context location may have guided their flavour decision – indeed the rats' preference for the congruous flavour would suggest that this was the case, in line with the conditioning account mentioned above.

It should be noted however that this possible awareness of an upcoming flavour choice during the learning trials is not the same as the ability to genuinely envisage the future and plan for it. The difference can be likened to that between episodic and semantic memory, with the former being genuine recall of a complete past event, and the latter a general awareness or knowledge, based on a previous experience that is not necessarily recalled. The distinction is illustrated by the study of King the gorilla (Schwartz, Hoffman & Evans, 2005). King was asked at a regular time at the end of each day to indicate using photographs the keeper that had attended to him earlier in the day, and the foodstuffs he had been provided with etc. King's performance was very good, which may have suggested an ability to genuinely recall the events he relayed. The authors concede however that the regular nature of the tests may have resulted in King storing the memories semantically - such that when he was tested, King simply 'knew' the answers rather than having to genuinely recall the events that had occurred that morning. Tapping into genuine episodic memory would have required the tests to be more sporadic, such that when asked, King would have to spontaneously actively recall what had happened to him previously. Similarly in the current experiment, an awareness of an upcoming flavour choice while the animals were still in context may mean that the flavour associated with this context is pre-selected as the flavour to be consumed. This could have created the preference for the congruous flavour seen in the learning, though not the testing, trials. It is also possible of course that a simpler process was at work to produce the congruous preference seen in the learning trials: it is possible that the context to which the animals were first exposed simply acts as a primer, such that when placed in the decision box with a choice of flavours, the animals consume the one that is associated with the context they just experienced.

Summary

The current group of rats did not demonstrate future orientated behaviour, i.e., behaviour in response to future rather than current motivations. The future motivation in question was satiation by an upcoming flavour. The test for this was an unexpected flavour choice offered just before an animal was exposed to the anticipated flavour. The flavour choice consisted of the flavour soon to be experienced (the congruous flavour) and an alternative (the incongruous flavour). An animal capable of anticipating satiation to the upcoming flavour should preferentially consume the *incongruous* flavour at choice, so as to maintain the pleasantness of the upcoming flavour. This would be consistent with the phenomenon of sensory specific satiety, whereby excessive consumption of one particularly flavour, smell, texture, etc. renders it subsequently less pleasant relative to other flavours, smells, textures, etc. (Rolls et al, 1981; 1983; 1991, and many others). However the animals' behaviour did not suggest an anticipation of upcoming satiety, because at choice the animals did not preferentially consume the incongruous over the congruous flavour. In fact there was no significant difference between the proportions of congruous and incongruous flavours consumed. The procedure was then altered in order to investigate if the animals were capable of *learning* to anticipate the upcoming satiation: instead of the flavour choice being offered unexpectedly, it was offered daily, such that the animals were able to learn from the flavour choices made in the decision box. This time however the animals consumed significantly more of the congruous flavour at choice. This suggested that the animals were not planning for a future state of satiation: an animal expecting to be satiated by a certain flavour in the near future would not eat that same flavour beforehand, as this would reduce the pleasantness of the upcoming flavour as consistent with the SSS literature. It is possible that the animals were genuinely anticipating exposure to a certain

flavour, but were not expecting to be satiated by it, and therefore were consuming the flavour congruous with their expectation. However it is equally plausible that the animals were able to anticipate the now frequent choices and were therefore being influenced by the context they experienced prior to the decision box. It is also possible that the animals were simply primed by the initial context to select the congruous flavour in the decision box.

In conclusion the animals in the present study did not solve this future planning task, nor did the animals learn to solve the task following repeated exposure to it. This is much the same result as obtained by Naqshbandi & Roberts (2006) who also used a repeated future planning task which produced a positive result in monkeys but not rats. There has been no study to date that has satisfactorily demonstrated the ability of rats to act on anticipated future states, as distinct from those currently being experienced. This may suggest that rats are simply incapable of this kind of future planning. However there are several reasons why it may be prudent to avoid such conclusions as yet: firstly, as aforementioned, the current study is subject to flaws such as assumptions relating to the learning of context sequences, and satiation, therefore a study that challenged these assumptions or used an alternative task may produce a positive result here. Secondly, the Naqshbandi & Roberts (2006) study required the animals to switch their innate preference from a larger pile of food to a smaller one, i.e., to act *against* current drives. While a successful result here would have been particularly compelling, it is not necessary for a future planning task to require action that could be considered currently counterproductive. This requirement of the Naqshbandi & Roberts experiment may have resulted in the task being too difficult for rats, and a similar task that did not include this requirement may be met with more success. Additionally, it should be noted that episodic-like memory *has* been demonstrated in

rats (Eacott, Easton & Zinkivskay, 2005; Eichenbaum and Fortin, 2003; Babb & Crystal, 2005), and there is extensive literature that supports the notion of a single Mental Time Travel System encompassing both episodic memory and future thinking (Atance & O'Neil, 2005; Suddendorf and Busby, 2005; Klein, Loftus & Kihlstrom, 2002; Hassabis, Kumaran, Vann & Maguire, 2007; Botzung, Denkova & Manning, 2008 and many others). Therefore a species that has one such capability (episodic-like memory) should also have the other (forward planning). Indeed, it is Tulving's (1999) view that the ability of an animal (human or nonhuman) to recall the past is useful only if it facilitates planning for the future. If rats *are* capable of recalling past episodes then it is reasonable to consider that this species can also plan for the future, even if a task has yet to be developed that allows a satisfactory behavioural demonstration of the ability.

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